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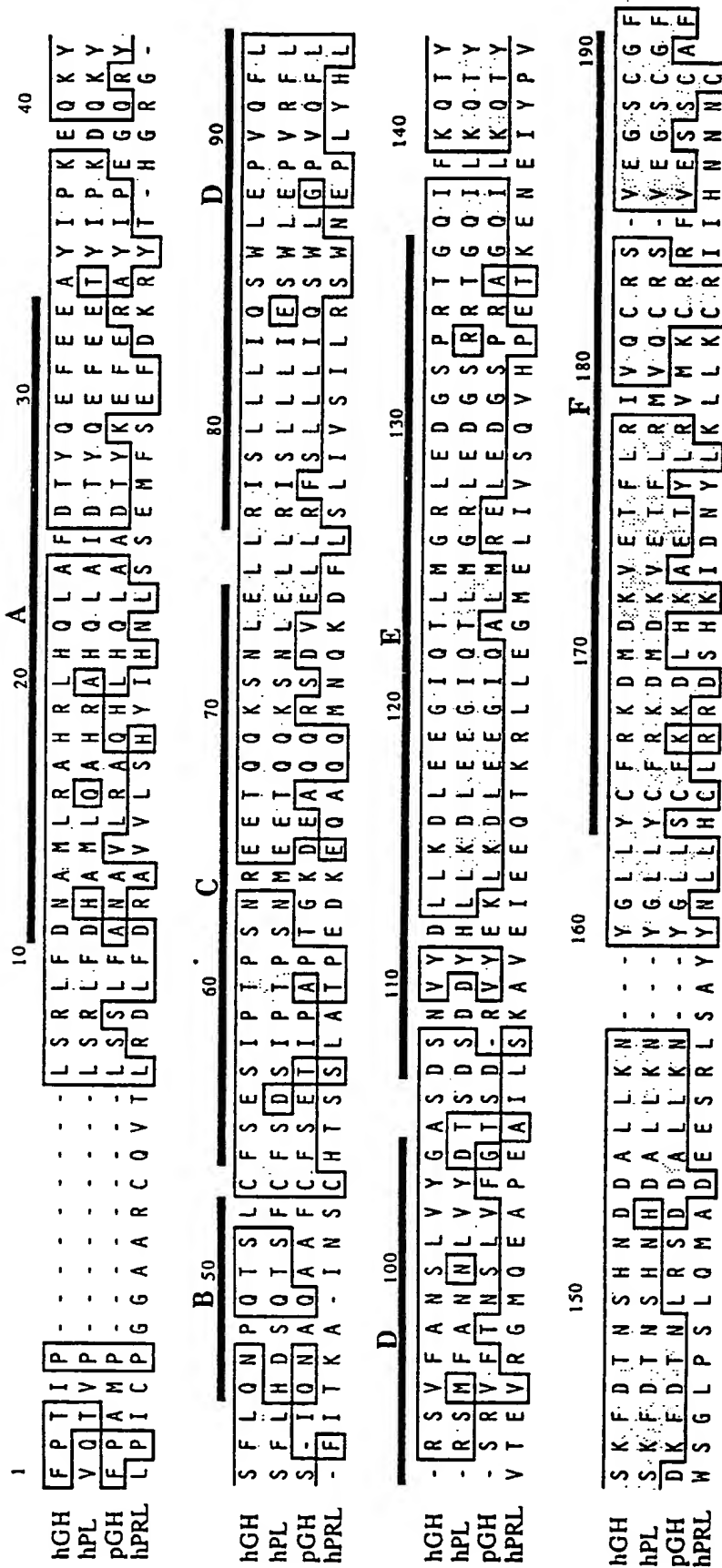


FIG.-2

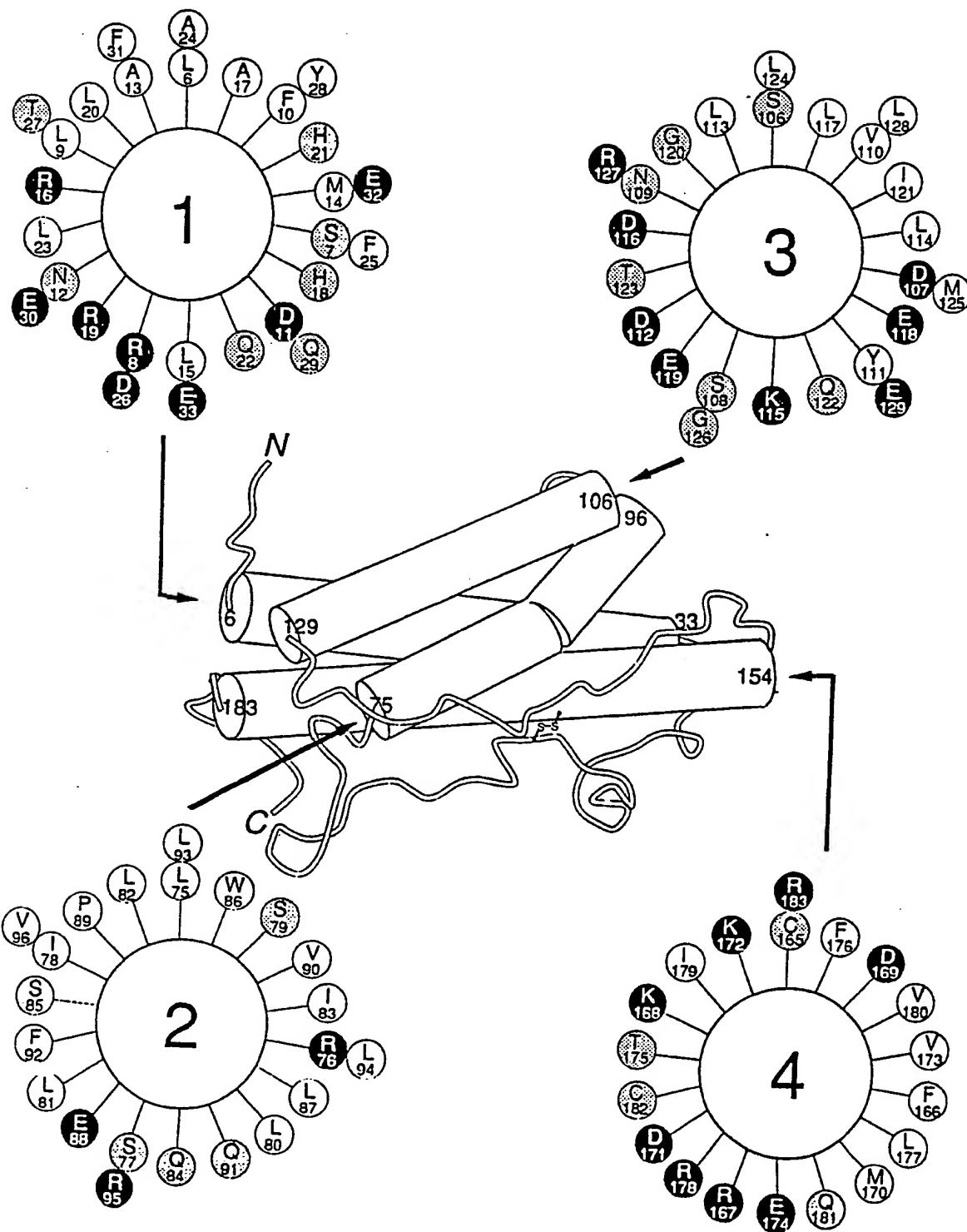


FIG.—3

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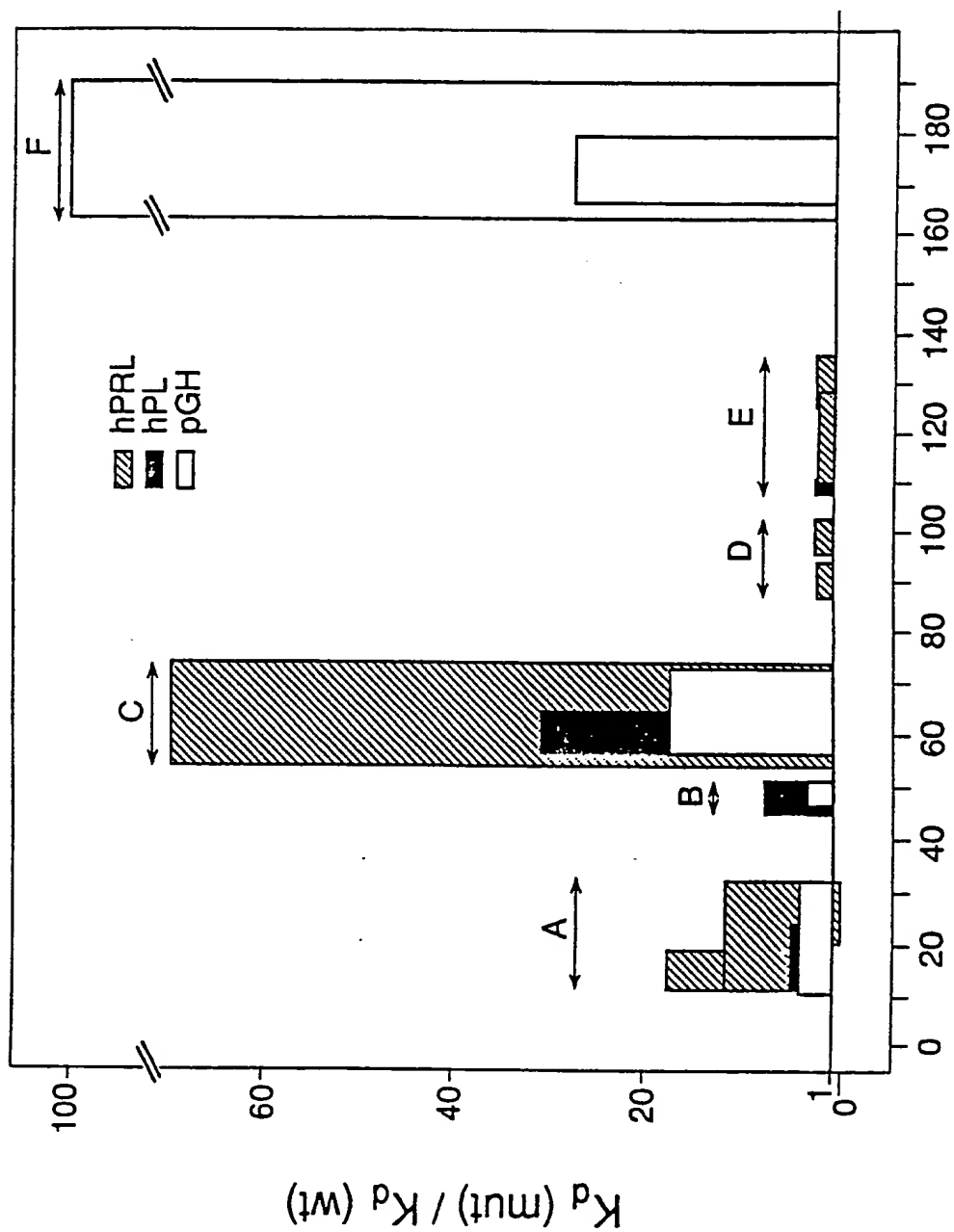


FIG.—4  
Residue number in hGH

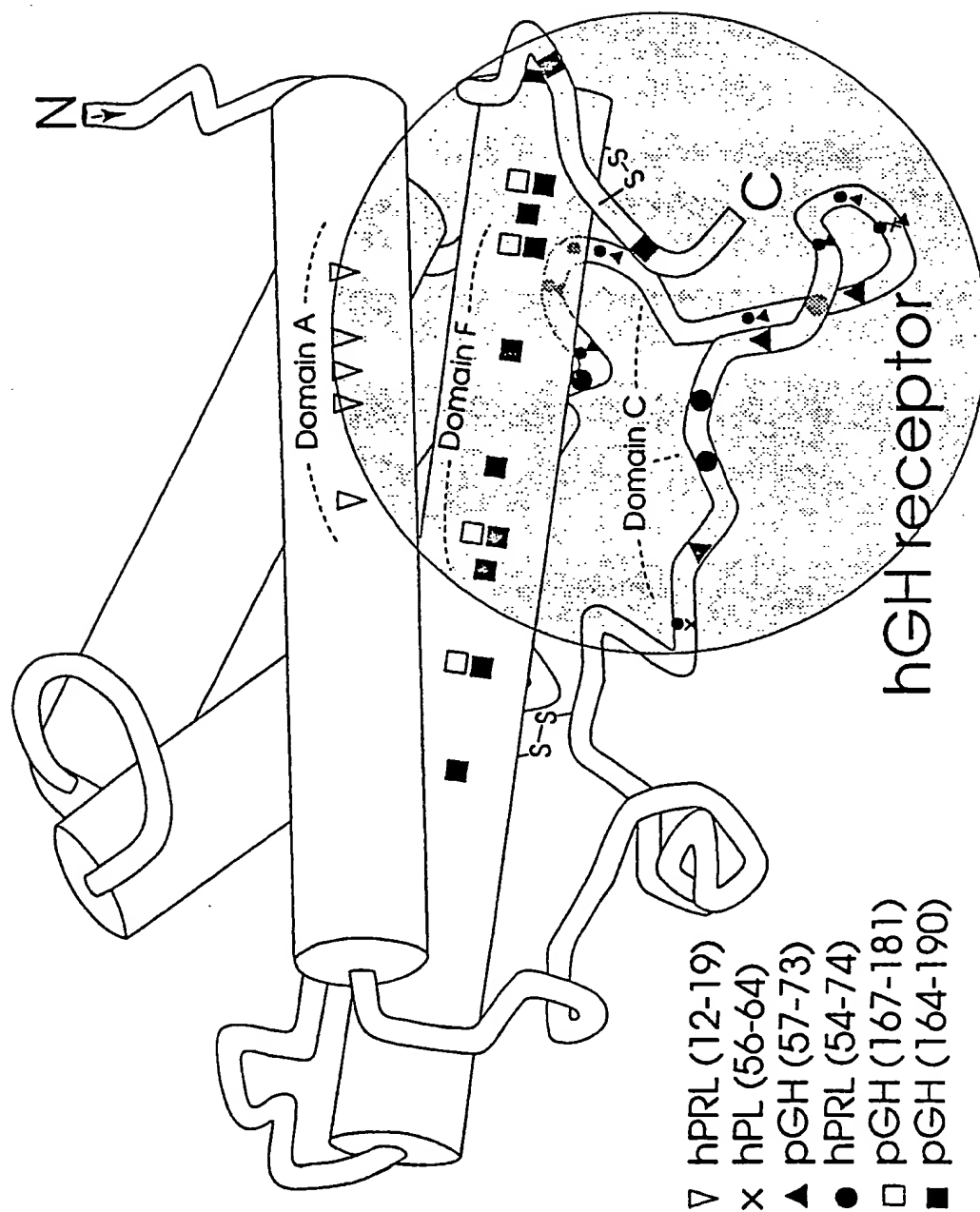


FIG.—5

- hPRL (88-95)
- ▲ hPRL (97-104)
- hPL (109-112)
- x hPRL (111-129) minus  
hPRL (126-136)

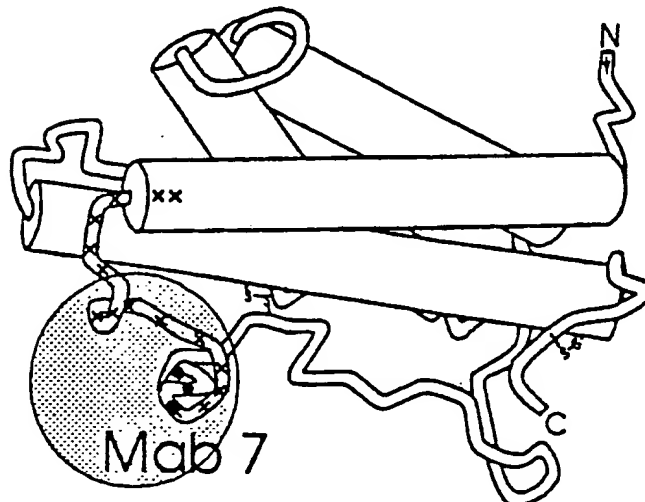
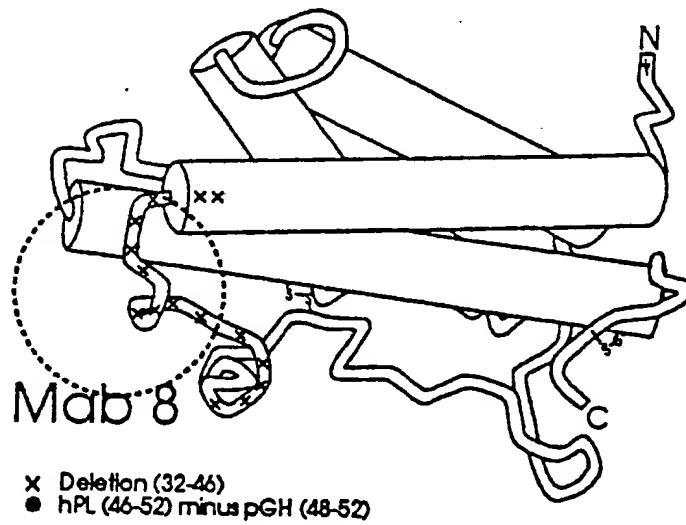
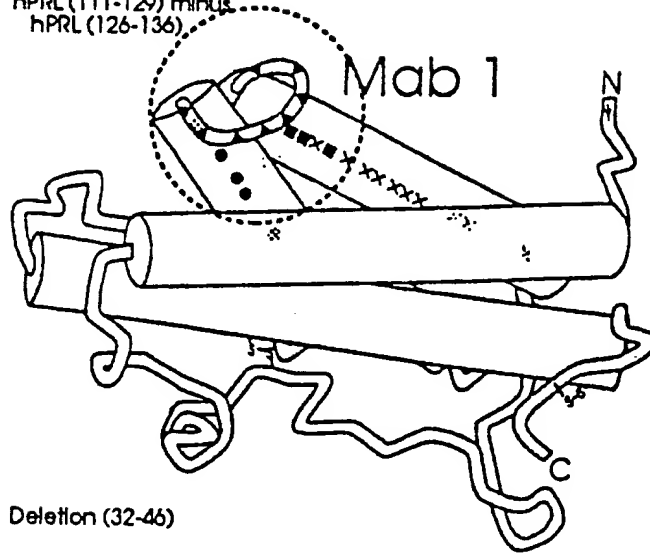
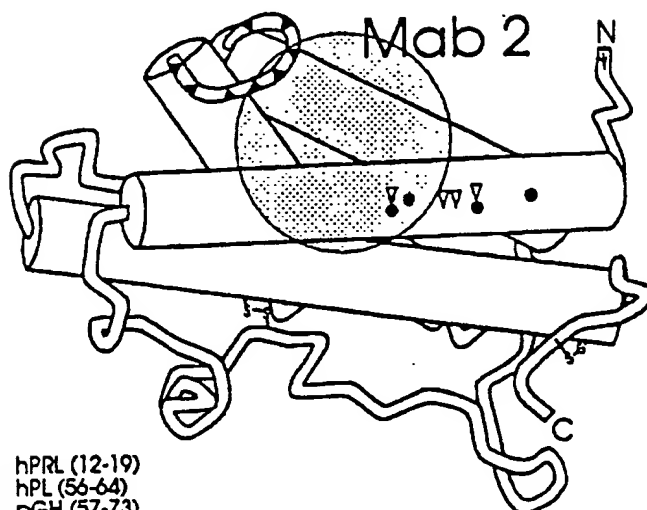


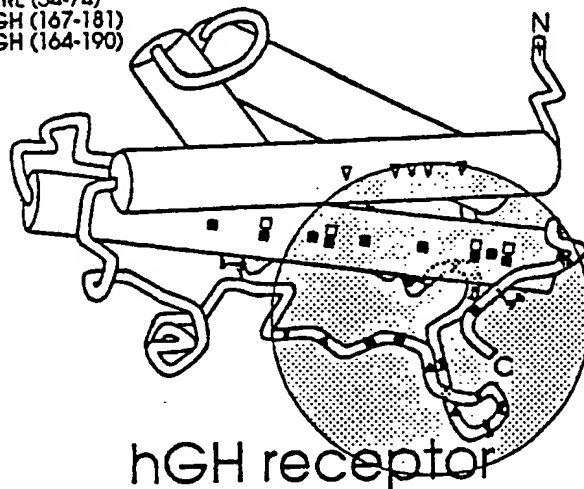
FIG.—6A

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- pGH (11-33) minus hPRL (22-33)
- ▽ hPRL (12-19) minus hPL (12-25)
- ▲ hPRL (97-104)



- ▽ hPRL (12-19)
- × hPL (56-64)
- ▲ pGH (57-73)
- hPRL (54-74)
- pGH (167-181)
- pGH (164-190)



- ▲ pGH (57-73) minus hPRL (54-74)
- pGH (164-190) minus pGH (167-181)

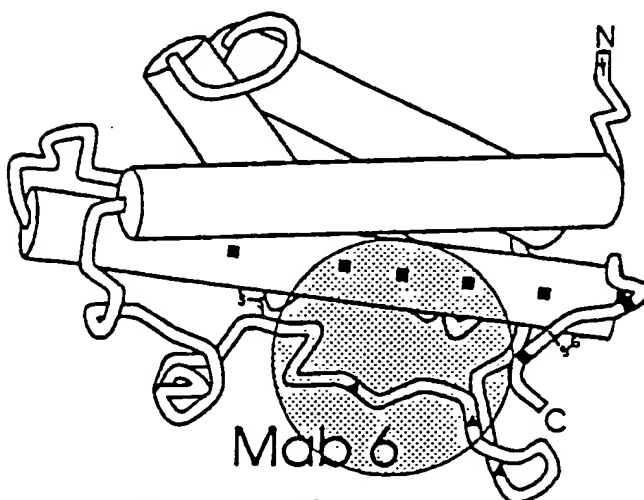
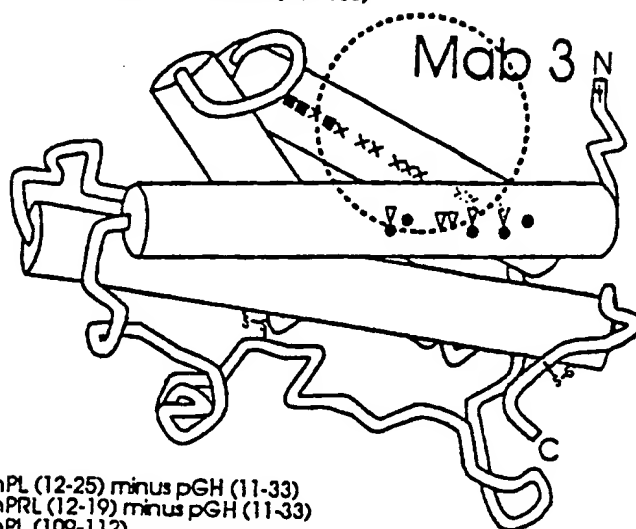


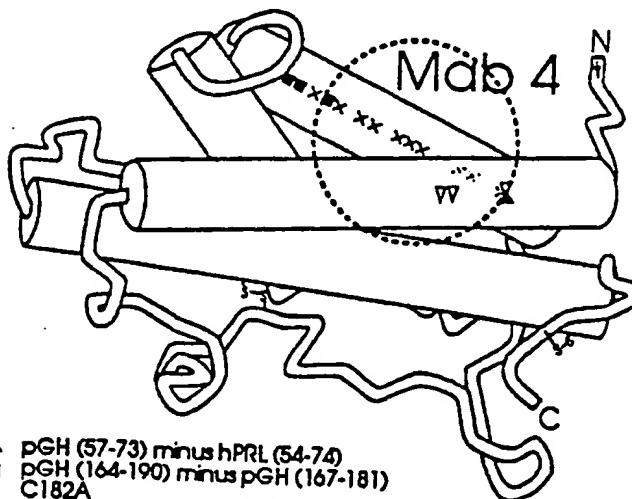
FIG.—6B

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- pGH (11-33) minus hPRL (22-33)
- ▲ hPL (12-25) minus hPRL (22-33)
- ▽ hPRL (12-79)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)



- ▲ hPL (12-25) minus pGH (11-33)
- ▽ hPRL (12-19) minus pGH (11-33)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)



- ▲ pGH (57-73) minus hPRL (54-74)
- pGH (164-190) minus pGH (167-181)
- C182A

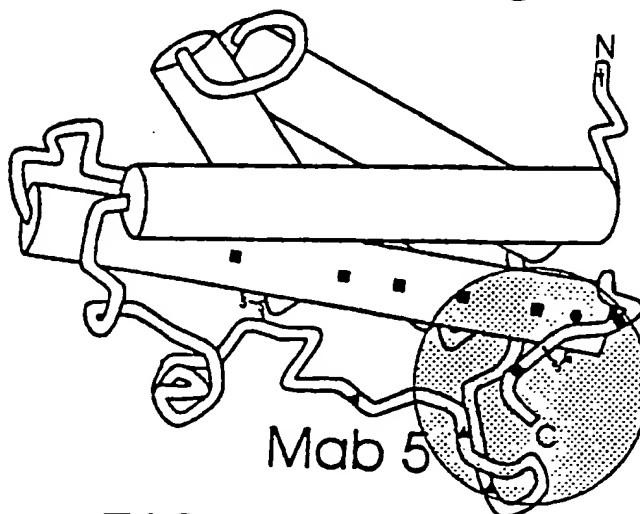
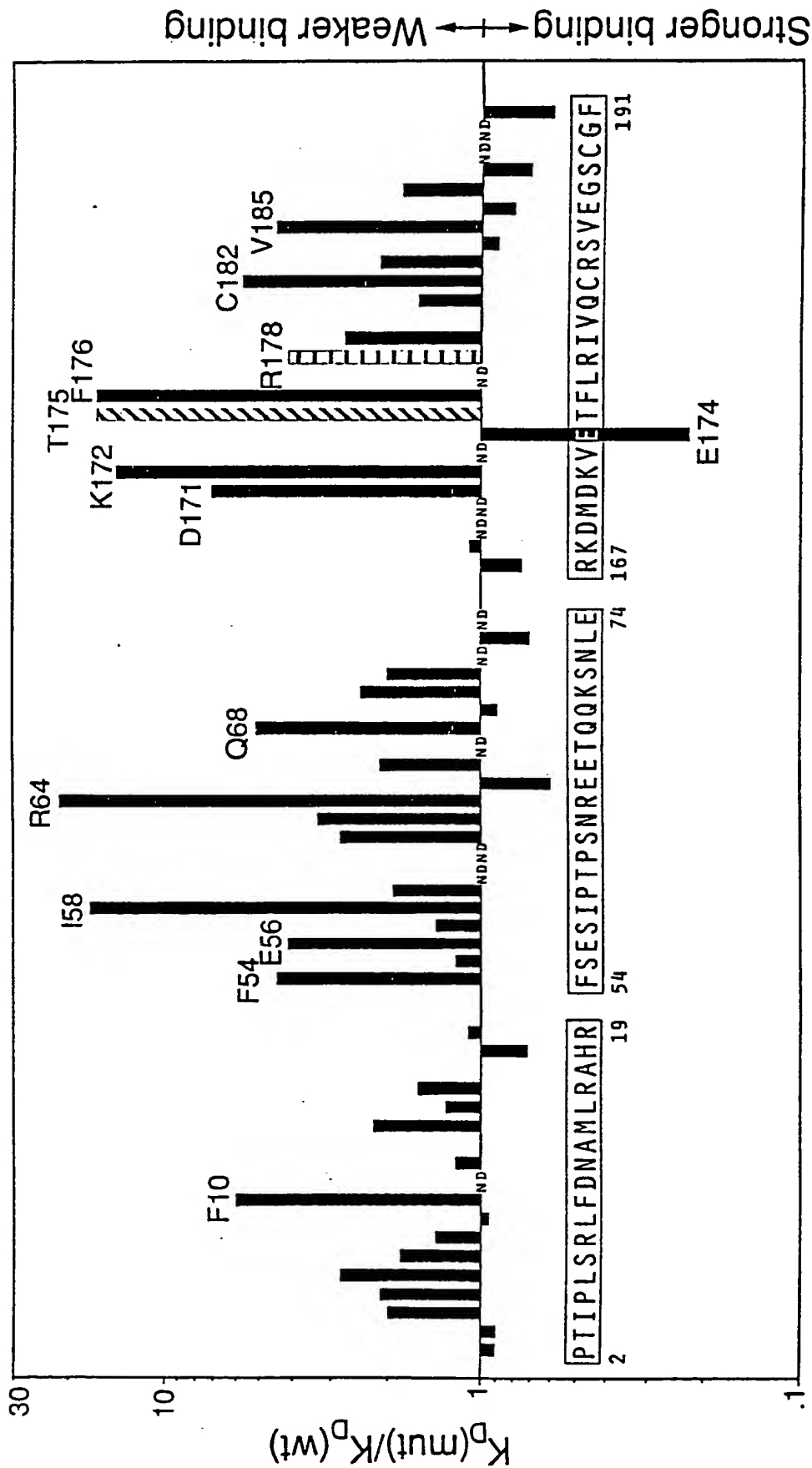


FIG.—6C

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## hGH Synthetic Gene

-20 Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser Ile Ala Thr Asn Ala Tyr Ala  
 ATG AAA AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT TTT TCT ATT GCT ACA AAT GCC TAT GCA  
 -10  
 -1  
 1  
 Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg Ala His Arg Leu His Gln Leu Ala  
 TTC CCA ACT ATA CCA CTA AGT CGA CTA TTC GAT AAC GCT ATG CTT CGG GCC CAT CGT CTT CAT CAG CTA GCC  
 20  
 79  
 Phe Asp Thr Tyr Gln Glu Phe Glu Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro  
 TTT GAC ACC TAC CAG GAG TTT GAA GAG GCC TAT ATC CCC AAG GAA CAG AAG TAT TCA TTC CTG CAG AAC CCC  
 40  
 142  
 StuI  
 50  
 Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln Lys Ser Asn  
 CAG ACC TCC CTC TGT TTC TCA GAA TCG ATT CCG ACA CCC TCC AAT CGC GAG GAA ACA CAA CAG AAA TCC AAC  
 60  
 70  
 214  
 ClaI  
 80  
 Leu Glu Leu Leu Arg Ile Ser Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val  
 CTA GAG CTC CTC CGC ATA AGC TTG CTG CTC ATC CAG TCG TGG CTC GAG CCC GTG CAG TTC CTG AGG AGT GTC  
 90  
 286  
 SacI  
 100  
 Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp Leu Leu Lys Asp Leu Glu Gly  
 TTC GCC AAC AGC CTG GTC TAC GGC GCC TCT GAT TCG AAC GTG TAC GAC CTG AAG GAC CTA GAG GAA GGG  
 110  
 120  
 358  
 NarI  
 130  
 Ile Gln Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser  
 ATC CAA ACG CTG ATG GGG AGG CTG GAA GAT GGC AGC CCG CGG ACT GGG CAG ATC TTC AAG CAG ACC TAC AGC  
 140  
 430  
 SacII  
 150  
 Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys Phe Arg Lys  
 AAG TTC GAC ACA AAC TCA CAC AAC GAT GAC GCA CTA CTC AAG AAC TAC GGG CTG CTC TAC TGC TTC AGG AAG  
 160  
 502  
 170  
 Asp Met Asp Lys Val Glu Thr Phe Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe AM\*  
 GAC ATG GAC AAG GTC GAG ACA TTC CTG CGC ATC GTG CAG TGC CGC TCT GTG GAG GGC AGC TGT GGC TTC TAG  
 180  
 574  
 MstI  
 190  
 PvuII

FIG.-8

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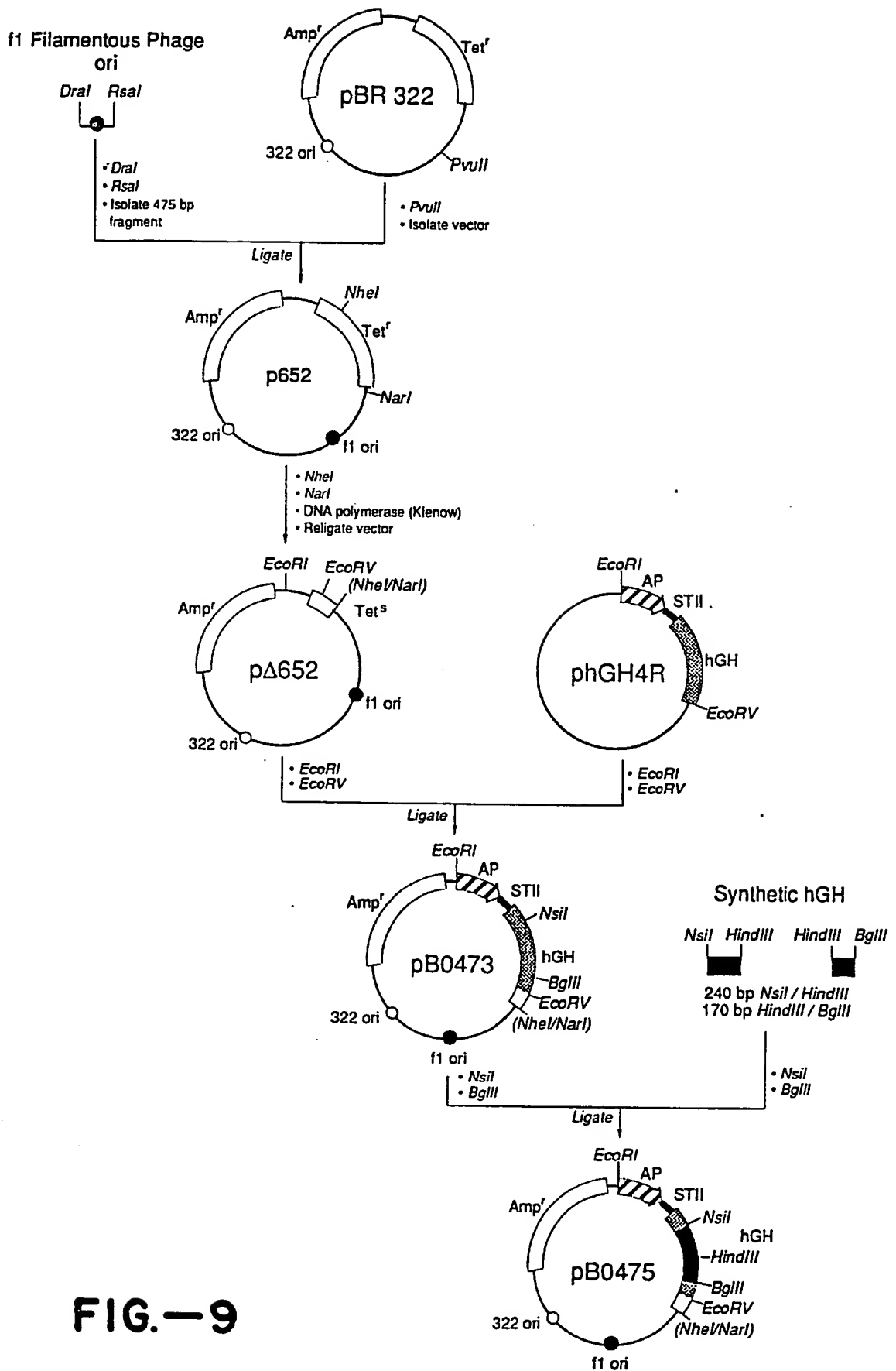


FIG.—9

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557 GCC TAT ATC CCC AAG GAA CAG AAG TAT TCA TTC CTG CAG AAC CCC CAG ACC TCC CTC TGT TTC TCA GAA TCG ATT CCG ACA
    CGG ATA TAG GGG TTC CTT GTC TAT ATA AGT AAG GAC GTC TTG GGG GTC TGG AGG GAG ACA AAG AGT CTT AGC TAA GGC TGT
    34 Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr
                                pstI
                                mnlI mnlI ddeI xnnI hinfi[M.taqI-]
                                clai taqI[M.claI-]
                                sstI
                                hgiJII
                                hgiAI[M.aluI-]
                                bsp1286[M.aluI-]
                                banII[M.aluI-]
                                aluI fnu4HI foki
                                hindII bbvI
                                bspI
                                taqI
                                xhoI
                                paeR7I
                                avai[M.taqI-]
639 CCC TCC AAT CGC GAG GAA ACA CAA CAG AAA TCC AAG CTA GAG CTC CTC CGC ATA AGC TTG CTG CTC ATC CAG TCG TGG CTC
    CGG AGG TTA CGC CTC CTT TGT TGT GTC TTT AGG TTT AGG TTT AGG TTT AGG TTT AGG TTT AGG TTT AGG TTT AGG TTT AGG TTT
    61 Pro Ser Asn Arg Glu Gln Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu Leu Ile Gln Ser Trp Leu
                                aluI
                                sstI
                                hgiJII
                                hgiAI[M.aluI-]
                                bsp1286[M.aluI-]
                                banII[M.aluI-]
                                aluI fnu4HI foki
                                hindII bbvI
                                bspI
                                taqI
                                xhoI
                                paeR7I
                                avai[M.taqI-]
719 GAG CCC GTG CAG TTC CTG AGG AGT GTC TTC GCC AAC AGC CTG CTC TAC GGC GCC TCT GAT TCG AAC GTG TAC GAC CTG CTG
    CTC GGG CAC GTC AAG GAC TCC TCA CAG AAG GAG TCG GAC ATG CCG CGG AGA CTA AGC TTG CAC ATG CTG GAC GAC
    88 Glu Pro Val Gln Phe Leu Arg Ser Val Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp Leu Leu
                                mnlI
                                ddeI
                                mstII
                                ecoRII
                                alwNI bsp36I
                                mboII
                                bstXI
                                bstNI accI
                                scrFI[dcM-]
                                ecoRII
                                hgiJII
                                hgiAI[M.aluI-]
                                bsp1286[M.aluI-]
                                banII[M.aluI-]
                                aluI fnu4HI foki
                                hindII bbvI
                                bspI
                                taqI
                                xhoI
                                paeR7I
                                avai[M.taqI-]
800 AAG GAC CTA GAG GAA GGG ATC CAA ACG CTG ATG GGG AGG CTG GAA GAT GGC AGC CCG CGG ACT GGC CAG ATC TTC AAG CAG
    TTC CTG GAT CTC CTT CCC TAG CTT CCC TAG CTT CCC TAG CTT CCC TAG CTT CCC TAG CTT CCC TAG CTT CCC TAG CTT CCC
    115 Lys Asp Leu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln
                                ppuMI
                                ecoO109I
                                mnlI
                                alwI
                                mboII
                                bstXI
                                bstNI accI
                                scrFI[dcM-]
                                ecoRII
                                hgiJII
                                hgiAI[M.aluI-]
                                bsp1286[M.aluI-]
                                banII[M.aluI-]
                                aluI fnu4HI foki
                                hindII bbvI
                                bspI
                                taqI
                                xhoI
                                paeR7I
                                avai[M.taqI-]
881 ACC TAC AGC AAG TTC TCG GAC ACA AAC TCA CAC GAT GAC GCA CTA CTC AAG AAC TAC GGC CTG CTC TAC TGC TTC AGG AAG
    TGG ATG TCG TTC AAG CTG TGT TTG AGT GTG TTT AGT GTG TTT AGT GTG TTT AGT GTG TTT AGT GTG TTT AGT GTG TTT AGT
    142 Thr Tyr Ser Lys Phe Asp Thr Asn Ser His Asn Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys Phe Arg Lys
                                taqI
                                hgiJII
                                hgiAI[M.aluI-]
                                bsp1286[M.aluI-]
                                banII[M.aluI-]
                                aluI fnu4HI foki
                                hindII bbvI
                                bspI
                                taqI
                                xhoI
                                paeR7I
                                avai[M.taqI-]

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FIG.-10B

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**FIG.-10D**



**FIG. -10F**





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sau3AI      mboI[dam-]      mboII[dam-]      sau3AI      mboI[dam-]
dpnI      xhoII      bstYI      xhoII      draI      mseI
3641 TTGATCTTTT CTACGGGGTC TGACGCTCAG TGAACGAAA ACTCAGCTTA AGGATTTTG      nlaIII      bspHI      alwI      hphI      bstYI      ahaIII
AACTAGAAA GATGCCCCAG ACTGCGAGC ACCTTGCTTT TGAATGCAAT TCCCTAAAAC CAGTACTCTA ATAGTTTTTC CTAGAAGTGG ATCTAGGAA
mboI[dam-]      sau3AI      mboI[dam-]      sau3AI      mboI[dam-]
dpnI      xhoII      bstYI      xhoII      draI      mseI
3741 TAAATTAATA ATGAGTGTAT AAATCAATCT AAAGTATATA TGAGTAACCT TGGTCTGACA GTTACCAATG      mseI      mnII      ddeI      dpnI
ATTAAATTTT TACITCAAAA TTAGTTAGA TTTCATATAT ACTCATTTGA ACCAGACTGT CAATGGTTAC      CTTAATCAGT GAGGCACCTA TCTCAGCGAT
AGATGCTGCTT ATCAACGGAC TGAGGGGCGC CACATCTATT GATGCTATGC CCTCCCGAAT GGTAGACCGG GGTACACGAG ITACTATGCG
sau96I[M.haeIII-]      sau96I      foki      pleI      hinfI
nlaIV      haeIII      fnu4HI      thal      fnuDII      bstUI
3841 CTGCTATTTT CGTTCATCCA TAGTTGCCGT AGTCCCGGTC GTGTAGATAA CTACGATPAG GGAGGGCTTA CCATCTGGCC CCAGIGCTGC AATGATACCG
GACAGATAAA GCAGGTAGGT ATCAACGGAC TGAGGGGCGC CACATCTATT GATGCTATGC CCTCCCGAAT GGTAGACCGG GGTACACGAG ITACTATGCG
sau96I[M.haeIII-]      sau96I      foki      pleI      hinfI
nlaIV      haeIII      fnu4HI      thal      fnuDII      bstUI
3941 CGAGACCCAC GCTCACCAGC TCCAGATTTA TCAGGATTTA TCAGCAATAA ACCAGCCAGC CGGAGGGCC GAGCGCAGAA GTGGTCTGTC AACTTTATCC GCCTCCATCC
GCTCTGGGTG CGAGTGGCGG AGTCTAAT AGTCTAAT AGTCTAAT TGGTGGGTG GCCTTCCCG GCCTTCCCG CTGCGCTCTT CACCAGGAG TTGAATAGG CGGAGGTAGG
sau96I[M.HI-]      pstI[M.HI-]
fnu4HI      bsvI      sfaNI
4041 AGTCTATTA TGTGTCGGG GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACCTTGTTGC ACCTTGTTGC GGCATCTGCA GGCATCTGCG TGTCACGCTC
TCAGATAATT AACACCGGCC CTTGATCTC ATTGATCAAG CGGICAAATA TCAACCGCT TGAACACAG GTAACGACGT CCGTAGCACC ACAGTGGCGG
sau3AI      mboI[dam-]      sau3AI      mboI[dam-]
mspi      hpaII      dpnI      mspl      hpaII      dpnI
4141 GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA TCACGGGAG TTACATGATC CCCCATGTTG TGCAAAAAG CGGTTAGTC aluI      aluI
CAGCAACCA TACCGAAGTA AGTCGAGGCC AGGCTTGCT AGTTCGCTC AATGACTACT GGGGTACAC ACCTTTTTT GCCATCTGAG GAAGCCAGGA

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FIG.-10H

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**FIG. 101**

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4741 GGAATATAAA CAAATAGGGG TTCCGGCGAC ATTCCCGGA AAGTGCCAC CTGACGTCTA AGAACCAT ATTATCATGA CATTAACCTA TAAAAATAGG
CTTTTATTT GTTATCCCC AAGGCGCGTG TAAAGGGCT TTTCACGGTG GACTGCAGAT TCTTTGGTAA TATAGTACT GTAATGGAT ATTTTATCC

          hinPI          ahaiI          nlaIII          msei
          hhai          acyl ddeI          bspHI
          thal          aaiI
          fnuDI
          nlaIV bstUI[M.hhai-]
4741 GGAATATAAA CAAATAGGGG TTCCGGCGAC ATTCCCGGA AAGTGCCAC CTGACGTCTA AGAACCAT ATTATCATGA CATTAACCTA TAAAAATAGG
CTTTTATTT GTTATCCCC AAGGCGCGTG TAAAGGGCT TTTCACGGTG GACTGCAGAT TCTTTGGTAA TATAGTACT GTAATGGAT ATTTTATCC

          sau96I[M.haeIII-]
          haeIII
          asuI
          ecoO109I
          mnlI          mboII
4741 CGTATCACGA GCCCTTTTCG TCTTCAA
GCATAGTGCT CCGGGAAGC AGAAGTT

>length: 4867

aarII(GACGTC):
accI(GTACAC):
accII(TCCGGA):
acyI(GRCGYC):
ahaII(GRCGYC):
ahaIII(TTTAA):
aluI(AGCT):
alwI(GGATC):
alwNI(CGNNNCTG):
apaI(GGGCCC):
apaLI(GTGCAC):
aseI(ATTAT):
asuI(GGACC):
asuII(TTCGAA):
avaI(CYCGRG):
avaII(GWCC):
avaIII(ATGCAT):
balI(TGGCCA):
bamHI(GGATCC):
banI(GGYRCC):
banII(GRCGYC):
bbvI(GCAGC):
bclI(dam-)(TGATCA):

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FIG.-10J

FIG. -10K

917 1277 1427 2041 2565 2688 3084 3662 4412  
hgai(GAGCC):  
677[M.aluI-] 1502 2798 3296 4457 4542  
767 1086 1129 1326 2374 3823  
hgici(GGYRCC):  
504 677 719 2408  
hgljii(GRGCCY):  
112 154 210 768 988 1111 1243 1394 1456 1492 1682 1765 2485 2493 2519 2541 2550  
2563 2583 2686 2716 2857 2890 3160 3227 3327 3501 3610 4003 4096 4433 4765  
112 154 210 768 988 1111 1243 1394 1456 1492 1682 1765 2485 2493 2519 2541 2550  
2563 2583 2686 2716 2857 2890 3160 3227 3327 3501 3610 4003 4096 4433 4765  
477[M.taqI-] 4414  
477 4414

71 691  
623[M.taqI-] 628[M.taqI-] 776[M.taqI-] 1341[M.hphI-] 1562[M.hphI-] 2068 2264  
2286 2882 2957 3353 3870  
1171 1180 1295 1321 1522 1702 1849 2109 2439 2569 2628 2662 3189 3336 3362 3552  
3956 3990 4057 4167 4409  
380 1136 1344 1565 2346 2592 2601 3726 3953 4349 4575 4590  
409 514 551 744 842 870[dam-] 1638 2465 2861 3632[dam-] 3723[dam-] 4478 4556[dam-]  
4665 4861  
139 817 868 1498 1705 2106 2572 3549 3624 3635 3643 3721 3733 3838 4179 4197  
4243 4501 4518 4554  
148 163 241 372 378 554 606 610 639 650 682 736 771 809 835 1013 1125 1185 1265  
1303 1330 1516 1830 1888 1944 2372 2579 2609 2871 3097 3154 3421 3821 3902 4032  
4238 4849  
69 257 324 1044 1066 1757 1979 2011 2125 2136 2148 2159 2176 2274 2545 2763  
3688 3740 3745 3759 3812 4047 4086 4451 4823  
1171 1180 1295 1321 1522 1702 1849 2109[M.bamHI-] 2439 2569[M.bamHI-] 2628 2662  
3189 3336 3362 3552 3956 3990 4057 4167 4409  
987 1393 1491 4095  
733  
1320 2438  
767  
1180 1295 1521 1849 2627 2662 3361 4057 4408  
2804  
523[M.aluI-] 1239  
40 964 1288 1495 1629 1854 1918 1983 2618 2723 2983 3703 4194 4204 4282 4318  
4711 4816  
504 767 816 1086 1129 1291 1326 1361 1475 1518 1797 2105 2374 2395 2407 2571  
3012 3051 3823 3917 3958 4169 4759  
646  
453  
1853 2617 2982  
716  
14 1352 1401  
2264 2286 2882 3353 3870  
801 1475 1517  
590 4116[M.HI-]

hincII(GATC):  
hindIII(GAATC):  
hpaII(CCGG):  
hphII(GGTGA):  
hmbIII(GAAGA):  
mboI(dam-)(GATC):  
mnmlI(CCIC):  
mpfI(TTA):  
mslI(TGCCCA):  
mstII(CCTNAGG):  
nmaeI(CCCGGC):  
ncfi(CCGCC):  
ndeI(CATATG):  
nhel(GCTAGC):  
nlaIII(CATG):  
nlaIV(GGNCCC):  
nrui(TCCCGA):  
nsiI(ATGCAT):  
nspCIx(RCATGY):  
paeRI(CTCGAG):  
pfml(CCANNNNTGG):  
pleI(GAGTC):  
ppumi(RGGWCY):  
pstI(CTGCAG):

**FIG.-10L**

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PvuI(CGATCG):
PvuII(CAGCTG):
PstI(GTAC):
PstII(GAGCTC):
SacI(CCGCGG):
SalI(GTCGAC):
SmaI(GATC):
Sau96I(GGATCC):
SmaI(AGTACT):
SbfI(CCSGG):
SbfII(dcm-)(CCWGG):
SfiI(GCATC):
SnaBI(TACGTA):
SpeI(ACTAGT):
SspI(AATATT):
SstI(GAGCTC):
SmaI(AGGCC):
SbfI(CCWGG):
SbfII(CTCGAG):
SmaI(RGATCY):
XmaIII(CGGCCG):
XmaI(GAANNNTTC):
not found:
AflII(CTTAAG), asp718(GGTACC), avrII(CCTAGG), bssHII(GCGCGC), bsteII(GGTNACC), espi(GCTNAGC), hpaI(GTTAAC),
kpnI(GGTACC), mluI(ACGGGT), ncoI(CCATGG), notI(GCGGCCG), rsrII(CGGWCCG), sfiI(GGCCNNNNNGGCC), smaI(CCCGGG),
sphI(GCATGC), xmaI(CCCGGG)
4242
270 1018[M.HI-I]
159 342 787 1174 2789 4354
677
854
477
139 817 868 1498 1705 2106 2572 3549 3624 3635 3643 3721 3733 3838 4179 4197
4243 4501 4518 4554
504[M.haeIII-] 505[M.haeIII-] 802 1182[M.haeIII-] 1297[M.haeIII-] 1476[dcm-]
1518 1797 1986[M.haeIII-] 2328[M.haeIII-] 3917[M.haeIII-] 3996[M.haeIII-] 4013
4235 4851[M.haeIII-]
4353
1180 1295 1521 1849 2627 2662 3361 4057 4408
541 757 1140 1479 3009 3130 3143
175 237 416 990 1144 1214 1458 1710 1719 1806 1884 1947 2658 2774 2829 2850
3070 4122 4332 4562
217
338
2127 4677
677
535
567 1406
478 486 626[M.claI-] 717 779 894 975 1305 2370 3082 4526
211 647 855 1271 1281 1426 1452 1574 1671 2043 2144 2520 2540 2564 2582 2584
2687 3028 3609 3939 4432 4764
968 2726
368
716
816 867 1704 2105 2571 3623 3634 3720 3732 4500 4517
290
623 2068 4470

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FIG.-10M

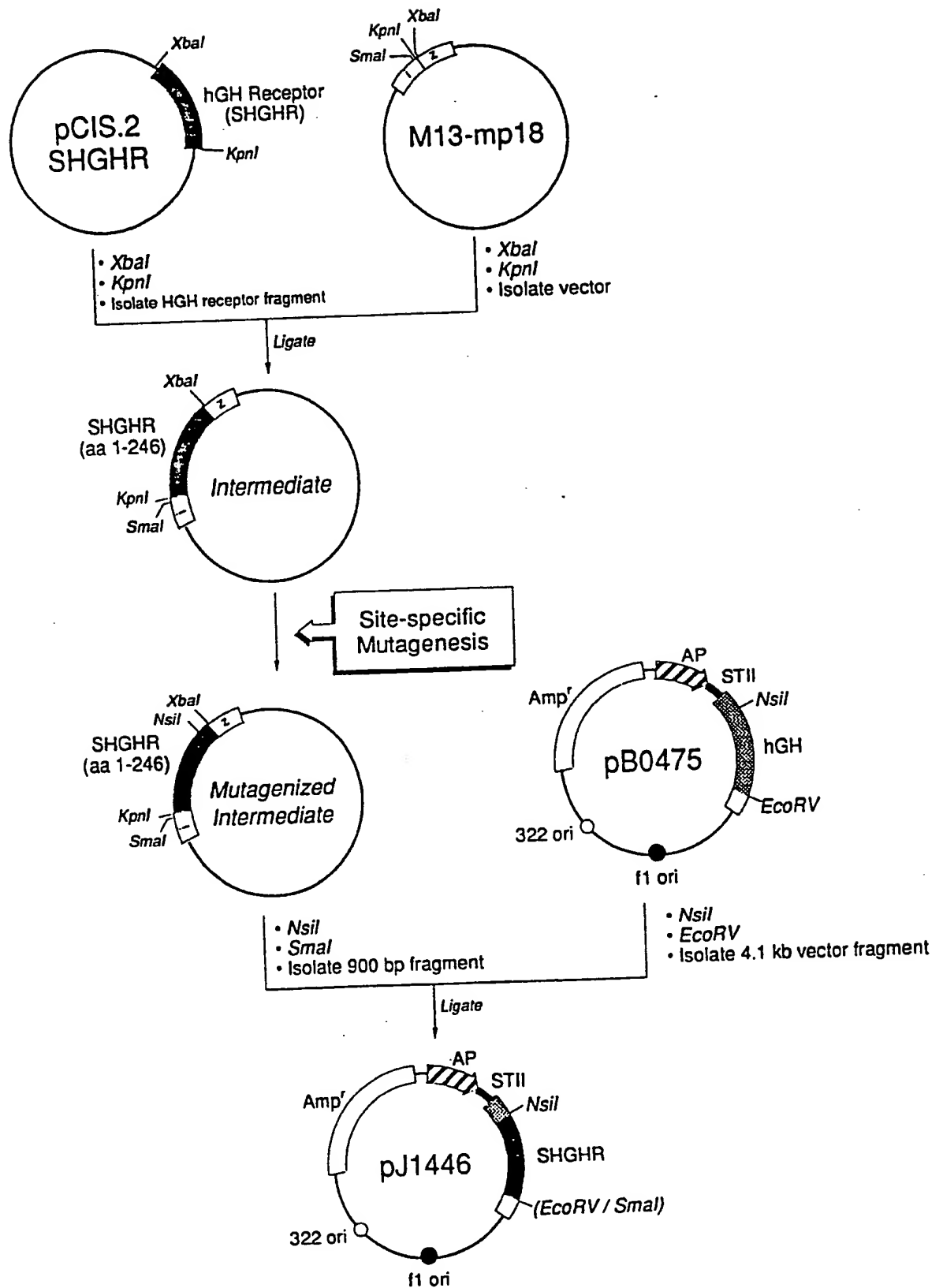


FIG.—II

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ecori pflmi nlaIII ddeI msei aluI hindIII  
1 GAATTCAACT TCTCCCAACT TTGGATAAGG AAATACAGAC ATGA AAAATC TCATTGCTGA GTTGTATTAT AAGCTTTGGA GATTATCGTC ACTGCAATGC  
CTTAAGITGA AGAGGPAIGA AACCTATTCC TTTATGCTG TACTTTTTAG AGTAACGACT CAACAATAA TTCGAACCT CTAATAGCAG TGACGTACC

hinPI hinPI hhaI bsmI  
101 TTCGCAATAT GGCGCAAAT GACCAACAGC GGTGATGA TCAGGTAGAG GGGCGCTCT ACAGGTAAA GCCCATGCC AGCATTCCTG ACAGCAGTAC  
AGCGTTATA CGCGTTTAA CTGGTTGTCG CCAACTAACT AGTCCATCTC CCCCGGACA TGCTCCATTT CGGCTACGG TCCTAAGGAC TGCTGCTATG

thai fnu4HI xmaII haeIII  
fnu4HI bsvI fnuDII xmaII  
fnu4HI bstUI[M.hhaI-] foki mnlI eagi  
bbvI hinPI bbvI hhaI snaBI sfanI msei aluI pvuII cfri bsmI  
201 GGAGCTGCTG CGCGATTACG TAAAGAAGT ATTGAAGAT CCTCGTCAGT AAAAAGTTAA TCTTTTCAAC AGCTGCATA AAGTTGTCTC GGCGAGACT  
CCTCGAGCAC CGGCTAATGC ATTTCTTCAA TAACCTTGTA GGAGCAGTCA TTTTTCATTT AGAAAGTTG TCGACAGTAT TTCAACAGTG CCGGCTCTGA

msei speI rsal xbaI mnlI hphi  
301 TAAGTTCGCT TTGTTTTTAT TTTTAACT ATTTGTAAT AGTACGCAAG TTCACGTAAG AAGGGTATCT AGAGTTGAG GTGATTTT  
ATATCAGCGA ACAAAAAATA AAAAATTACA TAAACATGA TCATGCGTTC AAGTGCAATT TTCCCATA TCTCCAATC CACTAAAA ATG AAA  
Met Lys Met Lys

nsII avallI haeIII hael mnlI  
395 AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT TTT TCT ATT GCT ACA AAT GCC TAT GGA TTT TCT GGA AGT GAG GCC  
TTC TTA TAG CGT AAA GAA GAA CGT AGA TAC AAG CAA AAA AGA TAA CGA TGT TTA CGG ATA CGT AAA AGA CCT TCA CTC CGG  
Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser Ile Ala Thr Asn Ala Tyr Ala Phe Ser Gly Ser Glu Ala

pleI hinfI haeIII stuI[dcM-]  
-21 scrFI[dcM-] hael  
scrFI[dcM-] ecorII ecorII ddeI mboII nlaIV  
hgAI ecorII msei bstNI  
bspl286 bstNI  
476 ACA GCA GCT ATC CTT AGC AGA CCC TGG AGT CTG CAA AGT GTT AAT CCA GGC CTA AAG ACA AAT AAT TCT TCT AAG GAG CCT  
TGT CGT CGA TAG GAA TCG TCT CGT GGG ACC TCA GAC GTT TCA CAA TTA GGT CCG GAT TTC TGT TTA AGA AGA TTT CTC GGA  
7 Thr Ala Ala Ile Leu Ser Arg Ala Pro Trp Ser Leu Gln Ser Val Asn Pro Gly Leu Lys Thr Asn Ser Lys Glu Pro

aluI fnu4HI bbvI ddeI  
476 ACA GCA GCT ATC CTT AGC AGA CCC TGG AGT CTG CAA AGT GTT AAT CCA GGC CTA AAG ACA AAT AAT TCT TCT AAG GAG CCT  
TGT CGT CGA TAG GAA TCG TCT CGT GGG ACC TCA GAC GTT TCA CAA TTA GGT CCG GAT TTC TGT TTA AGA AGA TTT CTC GGA  
7 Thr Ala Ala Ile Leu Ser Arg Ala Pro Trp Ser Leu Gln Ser Val Asn Pro Gly Leu Lys Thr Asn Ser Lys Glu Pro

**FIG.-12A**

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draIII  
 hphI  
 557 AAA TTC ACC AAG TGC CGT TCA CCT GAG CGA GAG ACT TTT TCA TGC CAC TGG ACA GAT GAG GTT CAT CAT GGT ACA AAG AAC  
 TTT AAG TGG TTC ACG GCA ACT GGA CTC GCT CTC TGA AAA AGT ACG GTG ACC TGT CTA CTC CAA GAT GAT His His Gly Thr Lys Asn  
 34 Lys Phe Thr Lys Cys Arg Ser Pro Glu Arg Glu Thr Phe Ser Cys His Trp Thr Asp Glu Val His His Gly Thr Lys Asn  
  
 sau96I  
 nlaIV  
 avaiI  
 asuI  
 puuMI  
 ecoO109I  
 638 CTA CGA CCC ATA CAG CTG TTC TAT ACC AGA AGG AAC ACT CAA GAA TGG ACT CAA GAA TGG AAA GAA TGC CCT GAT TAT GTT  
 GAT CCT GGG TAT GTC GAC AAG ATA TGG TCT TCC TGT TGA GTT CTT ACC TGA GTT Gln Glu Trp Thr Phe Ser Cys His Trp Thr Asp Glu Val His His Gly Thr Lys Asn  
 61 Leu Gly Pro Ile Gln Leu Phe Tyr Thr Arg Arg Asn Thr Gln Glu Trp Thr Phe Ser Cys His Trp Thr Asp Glu Val His His Gly Thr Lys Asn  
  
 aluI  
 pvuII  
 719 TCI GCT GGG GAA AAC AGC TGT TAC TTT AAT TCA TCG TTT ACC TCC ATC TGG ATA CCT TAT TGT ATC AAG CTA ACT AGC AAT  
 AGA CGA CCC CTT TTG TCG ACA ATG AAA TTA AGT AGC AAA TGG AGG TAG ACC TAT GGA ATA ACA TAG TTC GAT TGA ICG TTA  
 88 Ser Ala Gly Glu Asn Ser Cys Tyr Phe Asn Ser Ser Phe Thr Ser Ile Trp Ile Pro Tyr Cys Ile Lys Leu Thr Ser Asn  
 these first 9 bases differ from 262 and 265  
  
 aluI  
 pvuII  
 800 GGT GCT GGG GAA AAC AGC TGT TAC TTT AAT TCA TCG TTT ACC TCC ATC TGG ATA CCT TAT TGT ATC AAG CTA ACT AGC AAT  
 CCA CCA TGT CAC CTA CTT TTC ACA AAG AGA CAA CTA CTT TAT CAC GTT GGT CTA GGT GGG TAA CGG GAG TTG ACC TGA IGA AAT  
 115 Gly Thr Val Asp Glu Lys Cys Phe Ser Val Asp Glu Ile Val Gln Pro Asp Pro Pro Ile Ala Leu Asn Trp Thr Leu  
  
 foki  
 rsai  
 881 CTG AAC GTC AGT TTA ACT GGG ATT CAT GCA GAT ATC CAA GTG AGA TGG GAA GCA CCA CGC AAT GCA GAT ATT CAG AAA GGA  
 GAC TTG CAG TCA AAT TGA CCC TAA GTA CGT CTA TAG GTT CAC TCT ACC CTT CGT GGT GCG TTA CGT CTA TAA GTC TTT CCT  
 142 Leu Asn Val Ser Leu Thr Gly Ile His Ala Asp Ile Gln Val Arg Trp Glu Ala Pro Arg Asn Ala Asp Ile Gln Lys Gly  
  
 bsri  
 msei  
 962 TGG ATG GTT CTG GAG TAT GAA CTT CAA TAC AAA GAA GTA AAT GAA ACT AAA TGG AAA ATG ATG GAC CCT ATA TTG ACA ACA  
 ACC TAC CAA GAC CTC ATA CTT GAA GTT ATG TTT CTT CAT TTA CTT TGA TTT ACC TTT TAC TAC CTG GGA TAT AAC TGT TGT  
 169 Trp Met Val Leu Glu Tyr Glu Leu Gln Tyr Lys Lys Glu Thr Lys Trp Lys Met Met Asp Pro Ile Leu Thr Thr  
  
 foki  
 sau96I  
 nlaIV  
 avaiI  
 asuI  
 962 TGG ATG GTT CTG GAG TAT GAA CTT CAA TAC AAA GAA GTA AAT GAA ACT AAA TGG AAA ATG ATG GAC CCT ATA TTG ACA ACA  
 ACC TAC CAA GAC CTC ATA CTT GAA GTT ATG TTT CTT CAT TTA CTT TGA TTT ACC TTT TAC TAC CTG GGA TAT AAC TGT TGT  
 169 Trp Met Val Leu Glu Tyr Glu Leu Gln Tyr Lys Lys Glu Thr Lys Trp Lys Met Met Asp Pro Ile Leu Thr Thr

FIG.-12B

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```

sau3AI
mboI[dam-]
  dpnI
  alwI
xhoII
bstYI
1043 TCA GTT CCA GTG TAC TCA TTG AAA GTG GAT AAG GAA TAT GAA GTG CGT GTG AGA TCC AAA CAA CGA AAC TCT GGA AAT TAT
  AGT CAA GGI CAC AIG AGT AAC TTT CAC CTA TTC CTT ATA CTT CAC GCA CAC TCT AGG TTT GTT GCT TTG AGA CCT ATA ATA
196 Ser Val Pro Val Tyr Ser Leu Lys Ser Leu Lys Glu Tyr Glu Val Arg Val Arg Ser Lys Gln Arg Asn Ser Gly Asn Tyr

          bsrI rsal
          hgiAI
          bsp1286
          mnlI
1124 GCC GAG TTC AGT GAG CTC TAT GTA ACA CTT CCT CAG ATG AGC CAA TTT ACA TGT GAA GAA GAT TTC TAC TAG CG
  CCG CTC AAG TCA CTC CAC GAG ATA CAT TGT GAA GGA GTC TAC TCG GTT AAA TGT ACA CTT CTT CTA AAG ATG ATC GC
223 Gly Glu Phe Ser Glu Val Leu Tyr Val Thr Leu Pro Gln Met Ser Gln Phe Thr Cys Glu Glu Asp Phe Tyr AM*

          mseI
          hpaI
          hindII
          hincII
          thal
          fnuDII
          bstUI
          fnu4HI
1201 GCCGCGTTAA CTGTTTATT GCAGCTTATA ATGGTTACAA ATAAAGCAAT AGCATCACAA ATTTCACAAA TAAAGCATT TTTTCACTGC ATTCTAGTTG
  CGGCGCAATT GAACAAATAA CGTCGAATAT TACCAATGTT TATTTCGTTA TCGTAGTGT TAAAGTGTTT ATTTCGTAAA AAAAGTGACG TAAGATCAAC

          fnu4HI
          bsmI
          sau3AI
          mboI[dam-]
          dpnI
          alwI
          xhoII
          nlaIV
          bstYI
          bamHI
          nlaIII alwI
          fnu4HI
          nheI hinPI
          fnu4HI hhai
          bbvI haeII
1301 TGGTTTGTCC AACTCATCA ATGTATCTTA TCATGTCTGG ATCCCATCGT CCATTCCGAC AGCATCGCA GTCACTATGG CGTCTGCTA GCGCGGCCCT
  ACCAAACAGG TTGAGTAGT TACATAGAAT AGTACAGACC TAGGGTAGCA GGTAAAGCTG TCGTAGCGGT CAGTGATACC GCACGCGAT CGCGGCGGGA

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FIG.-12C





[illegible]

**FIG.-12F**

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FIG. -12G

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3401 GTATCTCAGT TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT  
 CATAGAGTCA AGCCACATCC AGCAAGCGAG GTTCGACCG ACACACGTGC GTGGGGGCA AGTCGGGCTG GCGACGGGA ATAGGCCATT GATAGCAGAA  
 hgiAI bsp1286 apalI aluI fnu4HI hpaII mspI hinPI hhaI fnu4HI bbvI  
 3501 GAGTCCACC CGGTAAGACA CGACTTATCG CCACCTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG CGGTGCTACA GACTTCTTGA  
 CTCAGGTTGG GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA TCGTCTCGCT CCATACATCC GCCACGATGT CTCAGAGAACT  
 mspI hpaII haeIII haeI hpaII mspI hinPI hhaI fnu4HI bbvI alwNI bsri mnlI  
 3601 GTGGTGCC TAACACTAGG TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC  
 TACCCACCG ATTGATGCC ATGTGATCTT CCTGTCTATA ACCATAGACG CGAGACGACT TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAACTAG  
 haeIII haeI hpaII mspI hinPI hhaI fnu4HI bbvI alwNI bsri mnlI  
 3701 CCGCAAACA ACCACCGCTG GTAGCGGTGG TTTTCTTGTG TGCAAGCGAG AGATTACGCG CAGAAAAAAG GGATCTCAAG AGATCCCTTT GATCTTTTCT  
 GCCGTTTGT TGGTGGCGAC CATCGCCACC CATCGCCACC AAAAAACAA ACGTTCGCTG TCTAATGCGC GTCTTTTCTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAAG  
 hpaII haeIII haeI hpaII mspI hinPI hhaI fnu4HI bbvI alwNI bsri mnlI  
 3801 ACGGGGTCTG ACGCTCAGTG GAACGAAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAAGG TCTTCACCTA GATCCCTTTA AATTAAAAAT  
 TGCCCCGAC TCGGAGTCAC CTGCTTTTG AGTGCAATTC CCTAAACCA GTACTCTAAT AGTTTTCTCT AGAAGTGGAT CTAGGAAAAA TTAATTTTAA  
 hgaI ddeI mspI hpaII haeIII haeI hpaII mspI hinPI hhaI fnu4HI bbvI alwNI bsri mnlI  
 3901 CAAGTTTAA ATCAATCTAA AGTATATATG AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTCA GGCACCTATC TCAGCGATCT GTCTATTTCG  
 CTTCAAAAAT TAGTTAGATT TCATATATAC TCATATATAC CAGACTGTCA ATGGTTAGCA ATTAGTCACT CCGTGGATAG AGTCGCTAGA CAGATAAAGC  
 mspI hpaII haeIII haeI hpaII mspI hinPI hhaI fnu4HI bbvI alwNI bsri mnlI

FIG.-12H



4001	TTCATCCATA AAGTAGGTAT	foki plei hinfi	GTTGCTGAC CAACGGACTG	TCCCCGTCGT AGGGGCAGCA	GTAGATAACT CATCTATTGA	ACGATACGGG TGCCGAATGG	ATCTGGCCCC TAGACCGGGG	AGTGCTGCAA TCACGACGTT	TGATACCGCG ACTATGGCGC	AGACCCACGC TCTGGGTGCG
4101	TCACCGGCTC AGTGGCCGAG	mspi hpaii hphi nlaiv tcccggtc agtgcccgag	caGATTTATC GTCTAAATAG	AGCAATAAAC TCGTTATTTG	CAGCCAGCCG GTCGGTCGGC	GARGGGCCGA CTTCCCGGCT	CGCGCTTCA GCGGCTTCA	CCAGACAGTT GAAATAGGCG	GAGGTAGGTC AGATATATTAA	
4201	GTTGCCGGGA CAACGGCCCT	scrfi ncii mspi hpaii cauii	alui AGCTAGAGTA AGCTAGCGGA	AGTAGTTCGC TCATCAAGCG	CAGTTAATAG GTCAATTATC	TTTGCAGCAAC AAACGCGTTG	GTTGTTGCCA CAACAACGGT	TTCTGTCAGG AACGACGTC	CATCGTGGTG GTAGCAACAC	CGTTTGGTAT GCAACACATA
4301	GGCTTCATTC CCGAGGTATG	mspi hpaii alui nlaiv agctccggtt ccgaggtatg	hpaii nlaiv agctccggtt ccgaggtatg	saui3AI mboi[dam-] dpmi dpmi	saui3AI mboi[dam-] dpmi dpmi	saui3AI mboi[dam-] dpmi dpmi	saui3AI mboi[dam-] dpmi dpmi	saui3AI mboi[dam-] dpmi dpmi	saui3AI mboi[dam-] dpmi dpmi	saui3AI mboi[dam-] dpmi dpmi
4401	AGAGTATAGT TCTTCATTCA	mspi hpaii hphi nlaiv tcccggtc agtgcccgag	caGATTTATC GTCTAAATAG	AGCAATAAAC TCGTTATTTG	CAGCCAGCCG GTCGGTCGGC	GARGGGCCGA CTTCCCGGCT	CGCGCTTCA GCGGCTTCA	CCAGACAGTT GAAATAGGCG	GAGGTAGGTC AGATATATTAA	
4501	AGTACTCAAC TCATGAGTTG	rsai scai ddei fnu4HI	caAGTCAATTC GTTTCAGTAA	AGTACTCAAC ACTCTTATCA	GTATGCGGCG CATACGCCGC	ACCAGGTTGC TGGCTCAACG	TCTTGGCCCG AGAACGGGCG	CGTCAACACG CGAGTTGTCG	GGATATATCC CGCCCATATA	GCAGAACTTT CGTCTTGTAA

**FIG. 12**

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1601	hgiAI bspl286 AAAGTGTCTC ATCAITGGAA ARCGTTCTCTC TTTTTCACGAG TAGTAACCTT TTGCAGAAG	bspl286 xmni mboII	GGGGCGARAA CTCTCAAGGA TCTTACCGCT GTTGACATCC CCCCGCTTTT GAGAGTTCTT AGAATGGCGA CAACTCTAGG	taqi bstyi xhoII	AGTTCGATGT AACCCACTCG TGCACCCCAAC TTCAGCTACA TTGGGTGAGC ACGTGGGTG
4701	hgiAI bspl286 TGAATTCAG CAICTTTTAC ACTAGAACTC GTAGAAATG	bspl286 xmni mboII	GGGGCGARAA CTCTCAAGGA TCTTACCGCT GTTGACATCC CCCCGCTTTT GAGAGTTCTT AGAATGGCGA CAACTCTAGG	taqi bstyi xhoII	AGTTCGATGT AACCCACTCG TGCACCCCAAC TTCAGCTACA TTGGGTGAGC ACGTGGGTG
4801	hgiAI bspl286 GAATACTCAT ACTCTTCCTT CTTAAGATA TGAAGAAGAA	bspl286 xmni mboII	GGGGCGARAA CTCTCAAGGA TCTTACCGCT GTTGACATCC CCCCGCTTTT GAGAGTTCTT AGAATGGCGA CAACTCTAGG	taqi bstyi xhoII	AGTTCGATGT AACCCACTCG TGCACCCCAAC TTCAGCTACA TTGGGTGAGC ACGTGGGTG
4901	hgiAI bspl286 AATAGGGGTT CCGCGCACAT TTATCCCAA GCGCGTGTA	bspl286 xmni mboII	GGGGCGARAA CTCTCAAGGA TCTTACCGCT GTTGACATCC CCCCGCTTTT GAGAGTTCTT AGAATGGCGA CAACTCTAGG	taqi bstyi xhoII	AGTTCGATGT AACCCACTCG TGCACCCCAAC TTCAGCTACA TTGGGTGAGC ACGTGGGTG
5001	hgiAI bspl286 CCCTTCGTC TTCAA GGGAAAGCAG AAGTT	bspl286 xmni mboII	GGGGCGARAA CTCTCAAGGA TCTTACCGCT GTTGACATCC CCCCGCTTTT GAGAGTTCTT AGAATGGCGA CAACTCTAGG	taqi bstyi xhoII	AGTTCGATGT AACCCACTCG TGCACCCCAAC TTCAGCTACA TTGGGTGAGC ACGTGGGTG

**FIG. -12J**

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aawi(GGATC): 851 1095 1339 1340 1852 2253 2254 2719 2720 3697 3771 3783 3868 3881 4345 4648  
 4666  
 aawi(CAGNNNCTG): 3541  
 apali(GTGCAC): 2946 3444 4690  
 asei(ATTAAT): 4194  
 asui(GGNC): 641 1024 1445 1624 1666 1945 2134 2476 4065 4144 4161 4383 4999  
 avai(CYCGRG): 1610  
 avaii(GGCC): 641 1024 1624[dcn-] 1666 1945 4161 4383  
 avaii(ATGCAT): 453  
 avaii(CCTAGG): 637  
 avaii(TGCCCA): 1629[dcn-]  
 bamhi(GGATCC): 1339 2253[M.mspi-] 2719[M.mspi-]  
 hani(GGYRCC): 1474 2522 3971  
 banii(GRGCYC): 2556  
 bbv-i(GCAGC): 204 207 479 1221 1384 1591 1615 1744 1747 1870 2664 2769 2866 3035 3472  
 3537 3540 3746 4074 4263 4440  
 138  
 bcli[dam-](TGATCA): 4137[M.haeIII-]  
 bgli(GCCNNNNGGC): 182 701 1289 1538  
 bsmi(GAATGC): 295 587 2779 4090 4855  
 bsmi(GTCTC): 495 1139 1650 2556 2946 3444 4605 4690  
 bsp1286(GDGGCHC): 3850 4858 4963  
 bsp1286(TCATGA): 1849 2256 2716  
 bsp1286(TCCGGA): 603 870 896 1049 1368 1695 1966 1990 2398 2877 2905 3533 3546 3663 4069 4187  
 bsri(ACTGG): 4230 4494 4669  
 bstni(CCWGG): 501 524 1627 3157 3278 3291  
 bstui(CGGC): 211[M.hhai-] 1203 1419 1429 1574 1600 1722 1819 2191 2292 2688[M.hhai-] 2688[M.hhai-]  
 2712[M.hhai-] 2730[M.hhai-] 2732[M.hhai-] 2835[M.hhai-] 3176 3757[M.hhai-] 4087  
 4580[M.hhai-] 4912[M.hhai-]  
 bstyi(RGATCY): 850 1094 1339 1852 2253 2719 3771 3782 3868 3880 4648 4665  
 cauII(CCSGG): 1443 1669 1997 2775 2810 3509 4205 4556  
 cfri(YGGCCR): 290 1199 1629 4411  
 ddei(CTNAG): 57 488 546 579 1158 1766 1928 2940 3405 3814 3980 4520 4946  
 dpni(GATC): 139 851 1095 1340 1646 1853 2254 2720 3697 3772 3783 3791 3869 3881 3986 4327  
 4345 4391 4649 4666 4702  
 3887 3906 4598  
 drai(TTTAAA): 562 2480  
 drai(CACNNNGTG): 290 1199 1629 4411  
 eaeI(YGGCCR): 290 1199  
 eagI(CGGCCG): 3008 4812  
 earI(CTCTTC): 640 1623[dcn-] 1665 4998  
 ecoO1091(RGGNCCY): 1  
 ecorI(GAATTC): 501 524 1627 3157 3278 3291  
 ecorI(CCWGG): 911  
 ecorI(GATATC): 911

FIG.-12K

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fnu4HI(GC:GC):  
 204 207 479 1198 1201 1221 1384 1393 1472 1591 1594 1601 1615 1744 1747 1870  
 1951 2664 2686 2700 2769 2866 2919 3035 3053 3056 3174 3329 3472 3537 3540 3746  
 4074 4263 4413 4440 4535 4764  
 fnu2II(CGCG):  
 211 1203 1419 1429 1574 1600 1722 1819 2191 2292 2668 2688 2712 2730 2732 2835  
 3176 3757 4087 4580 4912  
 238 811 959 963 1866 1955 2033 2194 2805 4003 4184 4471  
 1541 1639 4243  
 471 526 1629 3143 3154 3606  
 fspI(TGCGCA):  
 153 1390 1829 1912 2632 2640 3004 3374  
 haeII(WGGCCW):  
 291 472 527 1200 1446 1630 2134 2334 2476 3144 3155 3173 3607 4065 4145 4412  
 hacII(GGCC):  
 4999  
 1425 1575 2189 2713 2836 3232 3810 4560  
 hqaI(GACGCC):  
 495 1139 1650 2946 3444 4605 4690  
 1474 2522 3971  
 2556  
 hhvI(GCGC):  
 112 154 210 1391 1542 1604 1640 1830 1913 2633 2641 2667 2689 2698 2711 2731  
 2834 2864 3005 3038 3308 3375 3475 3649 3758 4151 4244 4581 4913  
 112 154 210 1391 1542 1604 1640 1830 1913 2633 2641 2667 2689 2698 2711 2731  
 2834 2864 3005 3038 3308 3375 3475 3649 3758 4151 4244 4581 4913  
 1206 4562  
 1206 4562  
 71  
 505 685 901 1489[M.hphI-] 1710[M.hphI-] 2216 2412 2434 3030 3105 3501 4018  
 1206  
 hpaI(GTAAAC):  
 1443 1469 1670 1850 1997 2257 2587 2717 2776 2810 3337 3484 3510 3700 4104 4138  
 4205 4315 4557  
 380 561 575 1492 1713 2494 2740 2749 3874 4101 4497 4723 4738  
 hphI(GGTGA):  
 409 542 1181 1184 1786 2613 3009 3780[dam-] 3871[dam-] 4626 4704[dam-] 4813  
 5009  
 mboI(GAAGA):  
 139 851 1095 1340 1646 1853 2254 2720 3697 3772 3783 3791 3869 3881 3986 4327  
 4345 4391 4649 4666 4702  
 mboI[dam-](GATC):  
 148 163 241 372 378 470 614 759 865 1136 1157 1413 1451 1478 1664 1978 2036  
 2092 2520 2727 2757 3019 3245 3302 3569 3969 4050 4180 4386 4997  
 69 257 324 519 744 893 1207 1905 2127 2159 2273 2284 2296 2307 2324 2422 2693  
 2911 3836 3888 3893 3907 3960 4195 4234 4599 4971  
 1443 1469 1670 1850 1997 2257[M.bamHI-] 2587 2717[M.bamHI-] 2776 2810 3337 3484  
 3510 3700 4104 4138 4205 4315 4557  
 1541 1639 4243  
 mstI(TGCGCA):  
 1468 2586  
 naeI(GCCGGC):  
 1443 1669 1997 2775 2810 3509 4205 4556  
 nciI(CCSGG):  
 2952  
 ndeI(CATATG):  
 1387  
 nheI(GCTAGC):

FIG.-12L

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nlaIII(CATG): 40 597 623 905 1176 1332 1436 1643 1777 2002 2066 2131 2766 2871 3131 3851 4342  
 4352 4430 4466 4859 4964  
 nlaIV(GGNNCC): 550 641 1024 1339 1439 1474 1509 1623 1666 1945 2253 2522 2543 2555 2719 3160  
 3199 3971 4065 4106 4317 4907  
 notI(GCGGCCGC): 1198  
 notII(ATGCCAT): 453  
 notIII(RCATGY): 1175 2001 2765 3130  
 notIV(CCANNNNTGG): 14 1500 1549  
 notV(GAGTC): 505 685 2412 2434 3030 3501 4018  
 notVI(RGGWCCY): 640 1623 1665  
 notVII(CTGCCAG): 4264[M.HI-]  
 notVIII(CGATCG): 4390  
 notIX(CAGCTG): 270 650 733  
 notX(GTAC): 159 342 627 804 1054 2937 4502  
 notXI(GATC): 139 851 1095 1340 1646 1853 2254 2720 3697 3772 3783 3791 3869 3881 3986 4327  
 notXII(GGNNCC): 4345 4391 4649 4666 4702  
 notXIII(M.HaeIII-) 641 1024 1445[M.HaeIII-] 1624[dcM-] 1666 1945 2134[M.HaeIII-] 2476[M.HaeIII-]  
 notXIV(M.HaeIII-) 4065[M.HaeIII-] 4144[M.HaeIII-] 4161 4383 4999[M.HaeIII-]  
 notXV(M.HaeIII-) 4501  
 notXVI(M.HaeIII-) 1443 1669 1997 2775 2810 3509 4205 4556  
 notXVII(M.HaeIII-) 501 524 1627 3157 3278 3291  
 notXVIII(M.HaeIII-) 175 237 416 1252 1362 1606 1858 1867 1954 2032 2095 2806 2922 2977 2998 3218  
 notXIX(M.HaeIII-) 4270 4480 4710  
 notXX(M.HaeIII-) 217  
 notXXI(M.HaeIII-) 338  
 notXXII(M.HaeIII-) 2275 4825  
 notXXIII(M.HaeIII-) 526[dcM-]  
 notXXIV(M.HaeIII-) 637 1554  
 notXXV(M.HaeIII-) 1453 2518 3230 4674  
 notXXVI(M.HaeIII-) 211 1203 1419 1429 1574 1600 1722 1819 2191 2292 2668 2688 2712 2730 2732 2835  
 notXXVII(M.HaeIII-) 3176 3757 4087 4580 4912  
 notXXVIII(M.HaeIII-) 2874  
 notXXIX(M.HaeIII-) 368  
 notXXX(M.HaeIII-) 850 1094 1339 1852 2253 2719 3771 3782 3868 3880 4648 4665  
 notXXXI(M.HaeIII-) 290 1199  
 notXXXII(M.HaeIII-) 2216 4618  
 not found:

aflIII(CTTAAG), apaI(GGGCCC), asp718(GGTACC), asuII(TTCGAA), bglII(AGATCT), bspMI(ACCTGC), bssHII(GCGCGC),  
 bstBI(TTCGAA), bstEII(GGTNACC), bstXI(CCANNNNTGG), bss36I(CCTNAGG), clai(ATCGAT), eco8I(CCTNAGG), ecoNI(CCTNNNNNAGG),  
 espI(CGTNAGG), kpnI(GGTACC), mluI(ACGGCT), mstII(CCTNAGG), nari(GGGGCC), ncoI(CCATGG), nruI(TCGCA), pae7I(CTCGAG),  
 rsiI(CGGWCCG), sacI(GAGCTC), sacII(CCGCGG), salI(GTCGAC), sfiI(GGCCNNNNNGGCC), smaI(CCCGGG), sphi(GCATGC),  
 sstI(GAGCTC), xhoI(CTCGAG), xmaI(CCCGGG)

FIG.-12M

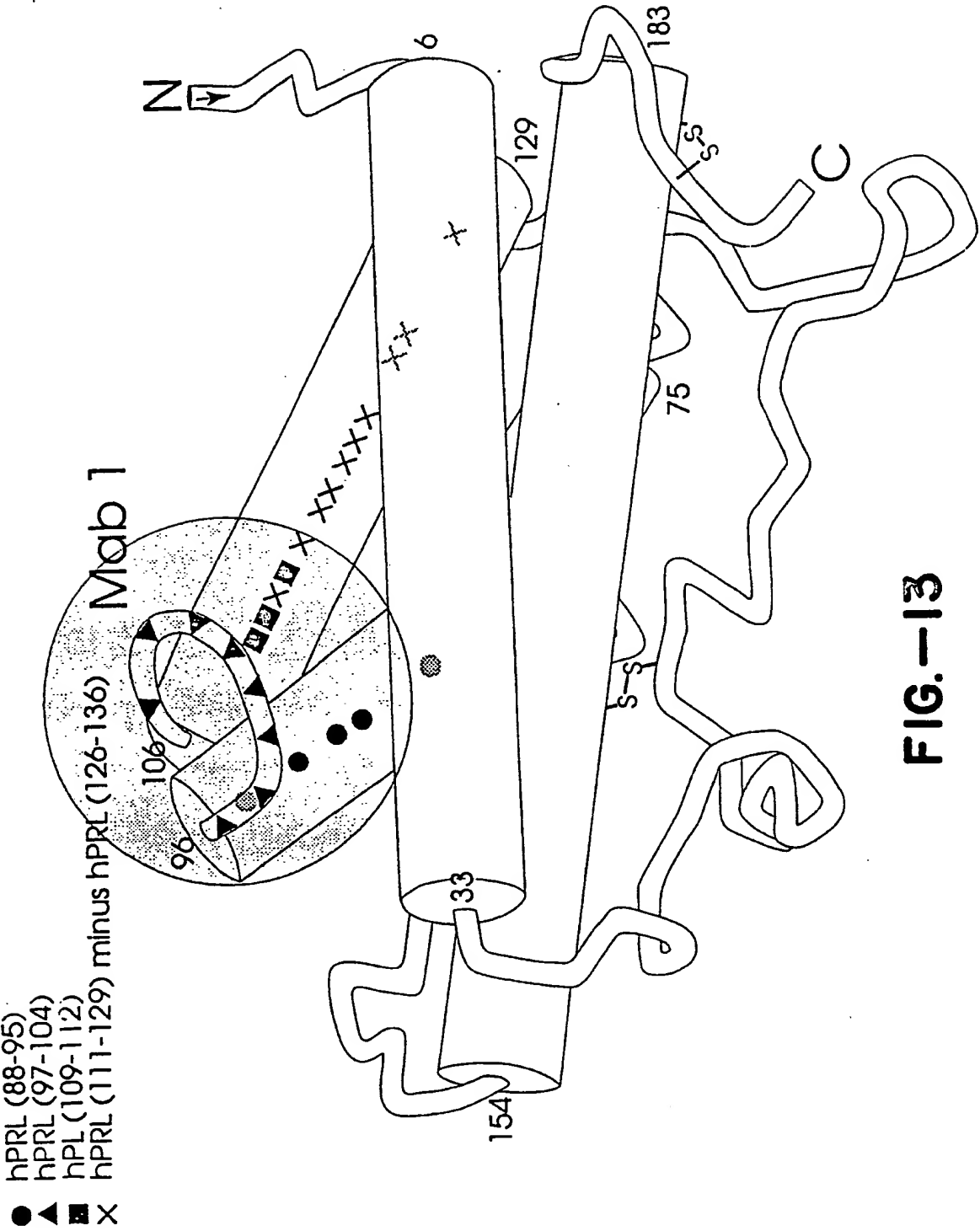


FIG.-13

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- pGH (11-33) minus hPRL (22-33)
- ▽ hPRL (12-19) minus hPL (12-25)
- ▲ hPRL (97-104)

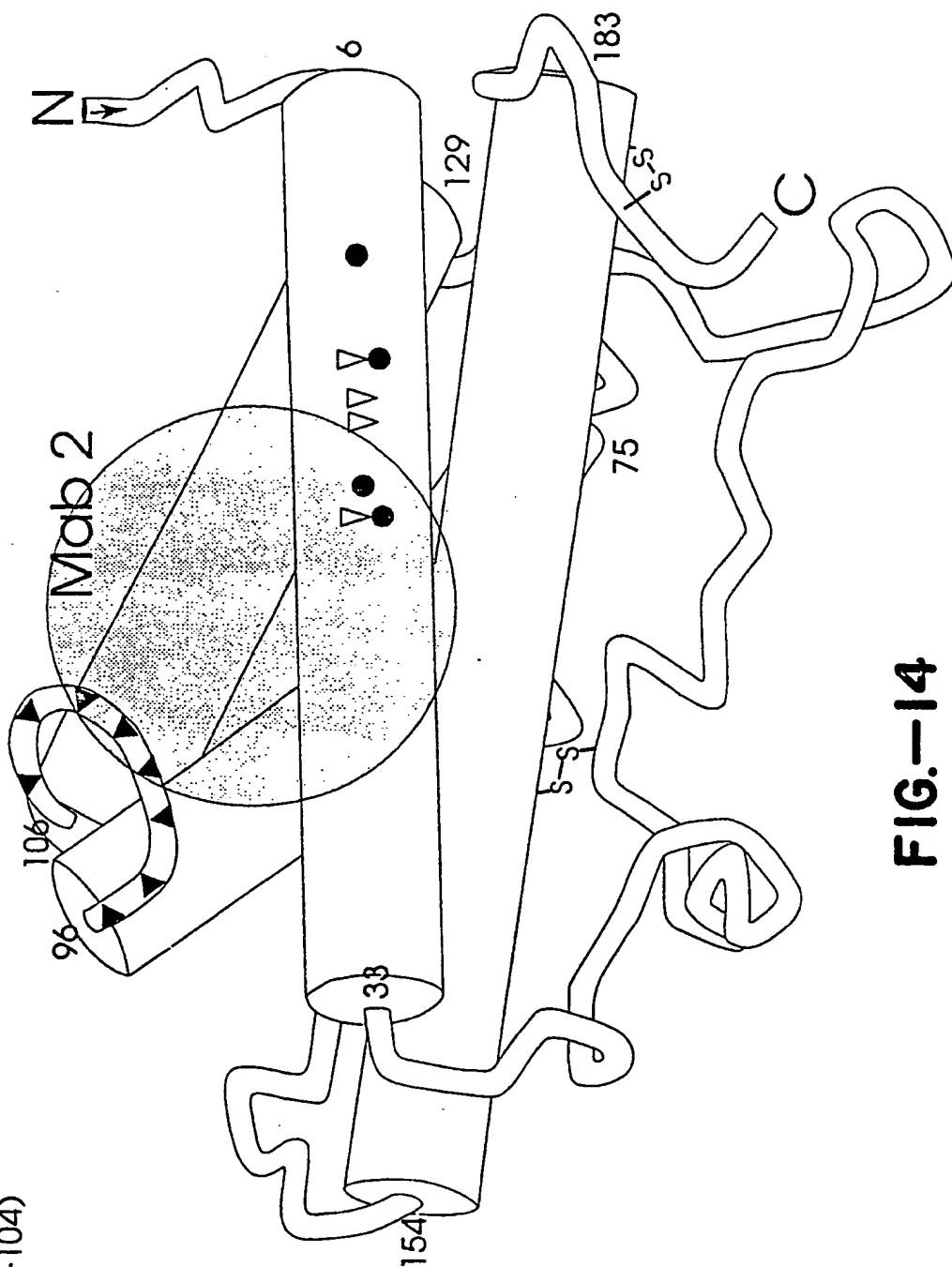


FIG.-14

- pGH (11-33) minus hPRL (22-33)
- ▲ hPL (12-25) minus hPRL (22-33)
- ▽ hPRL (12-79)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)

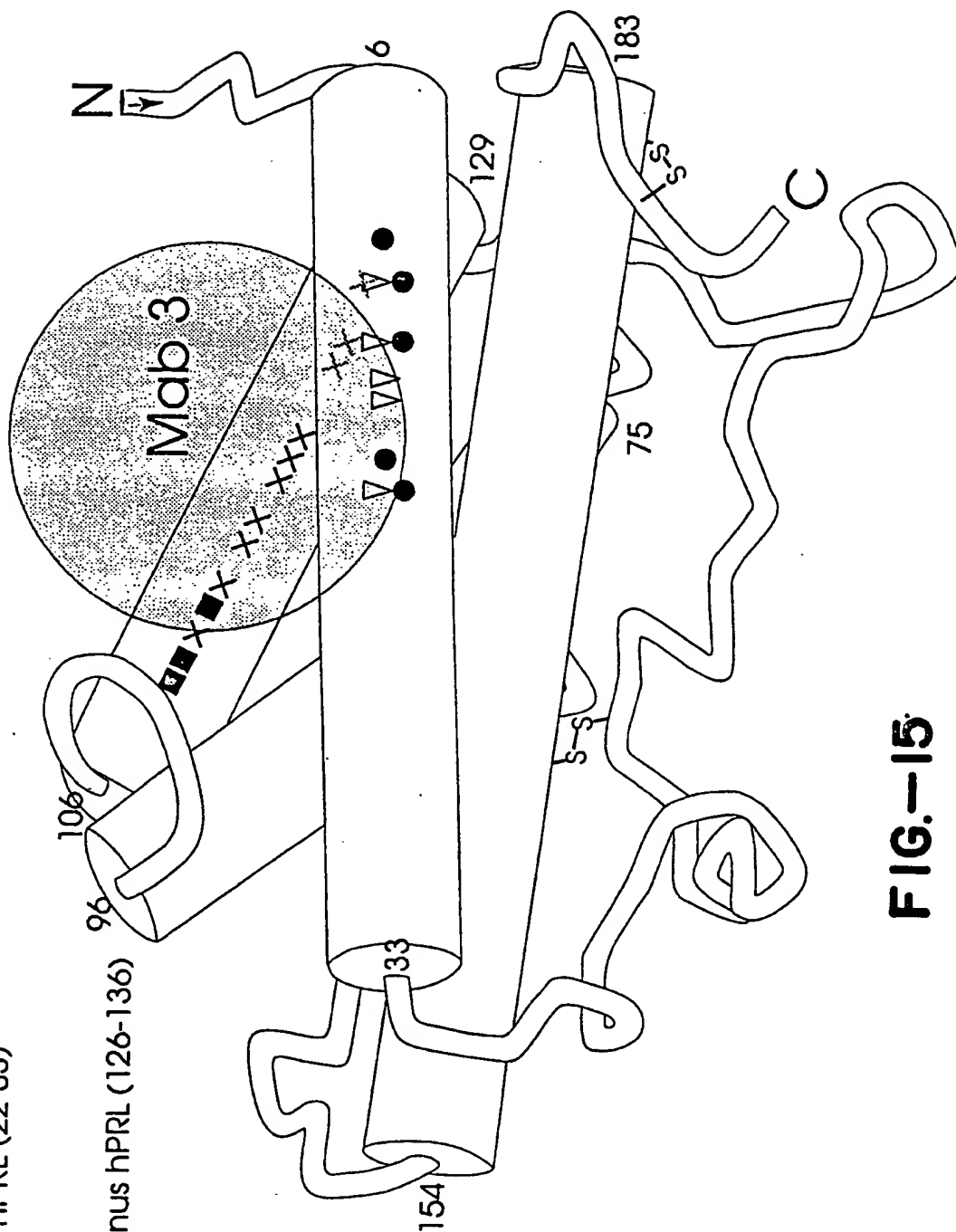


FIG.-15



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- ▲ hPL (12-25) minus pGH (11-33)
- ▽ hPRL (12-19) minus pGH (11-33)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)

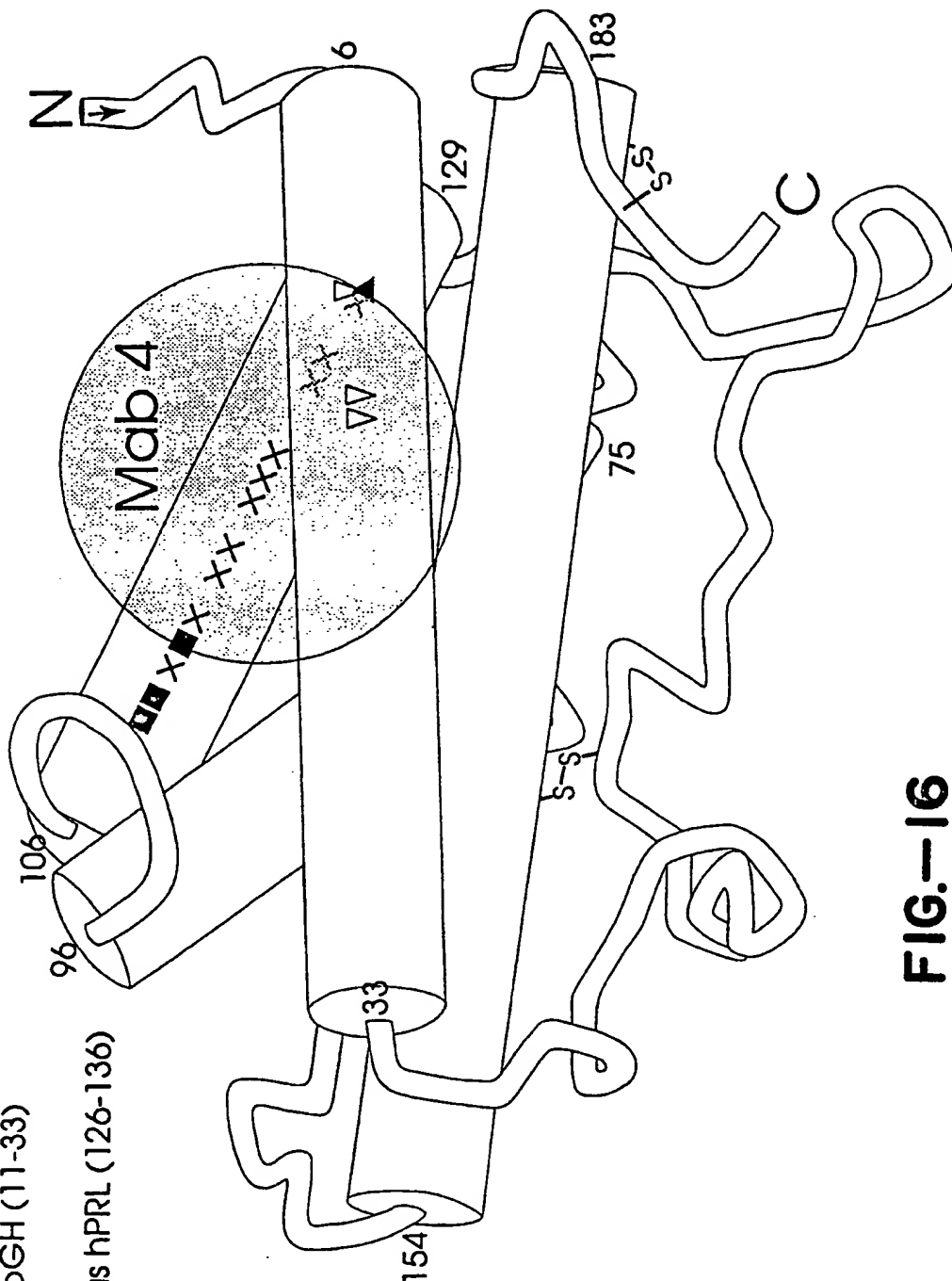


FIG.-16

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- ▲ pGH (57-73) minus hPRL (54-74)
- pGH (164-190) minus pGH (167-181)
- C182A

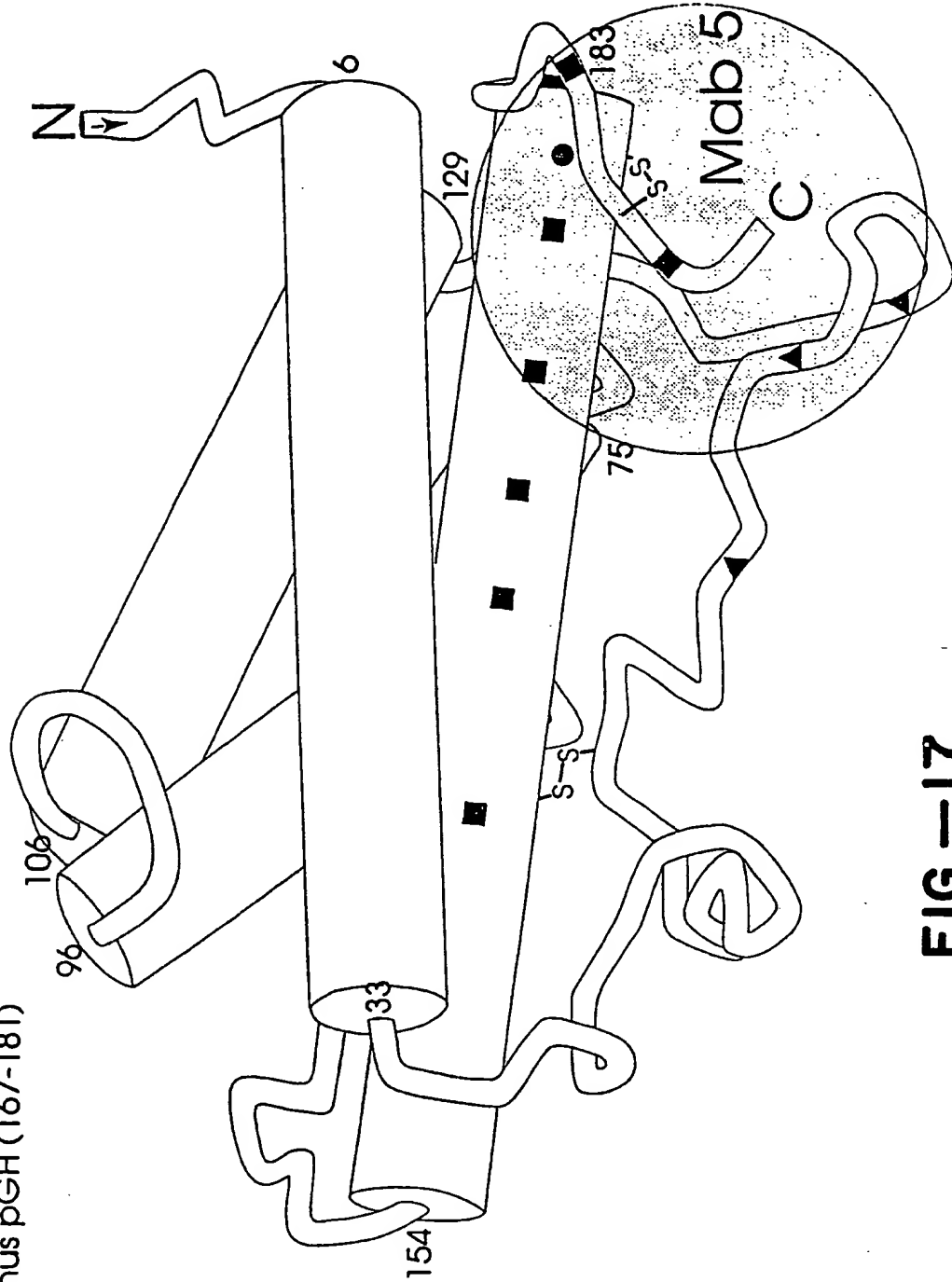
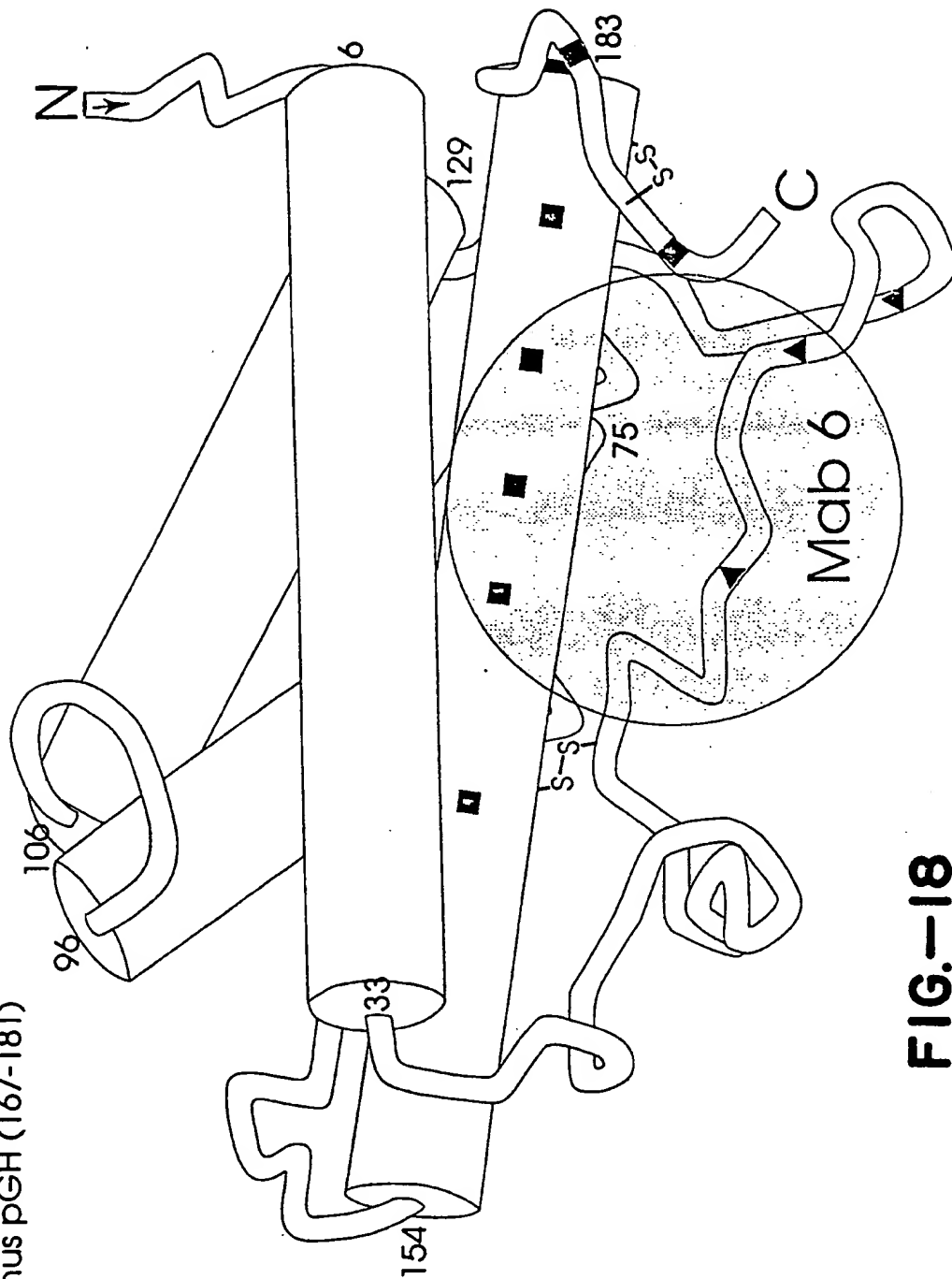
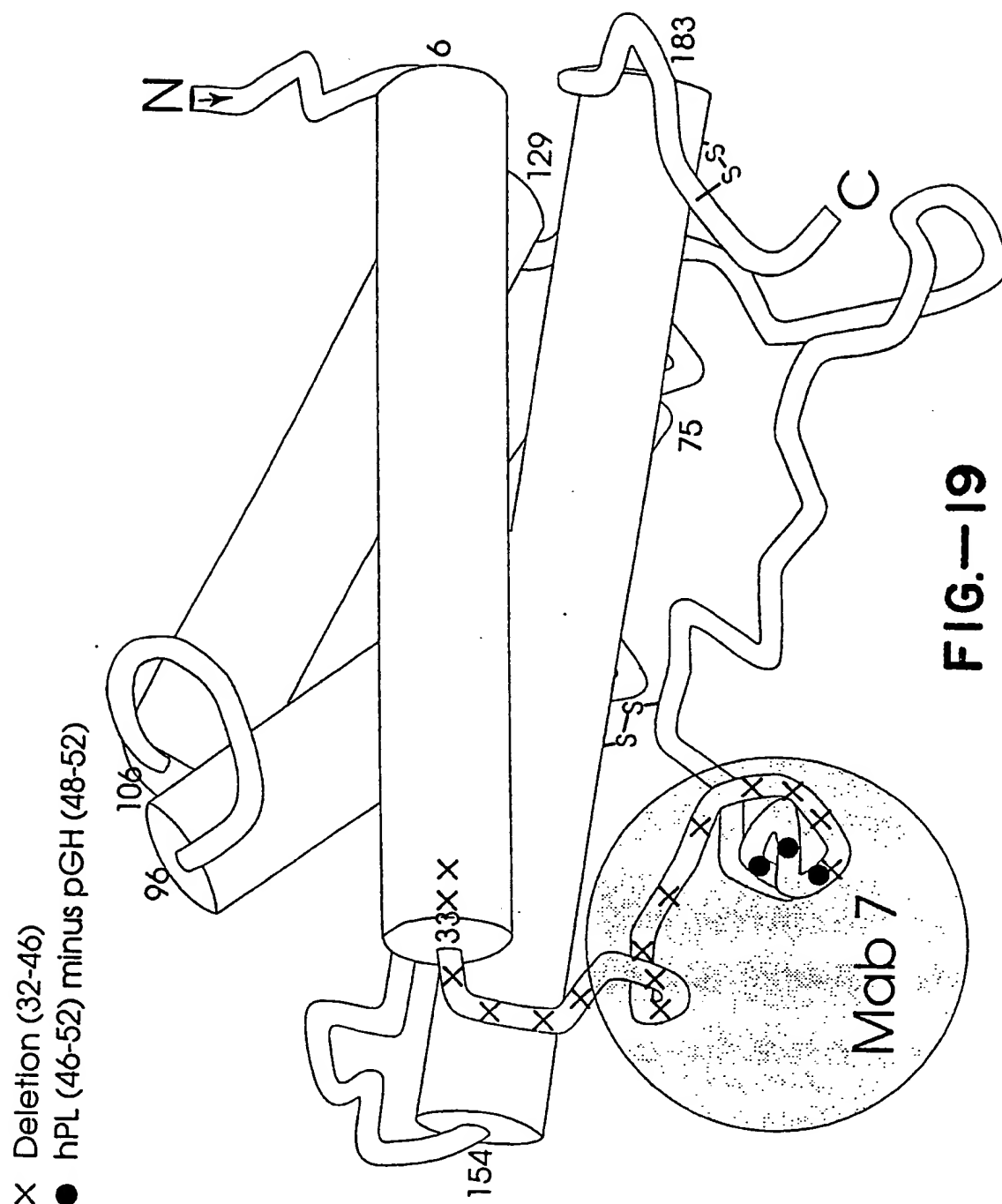


FIG.-17



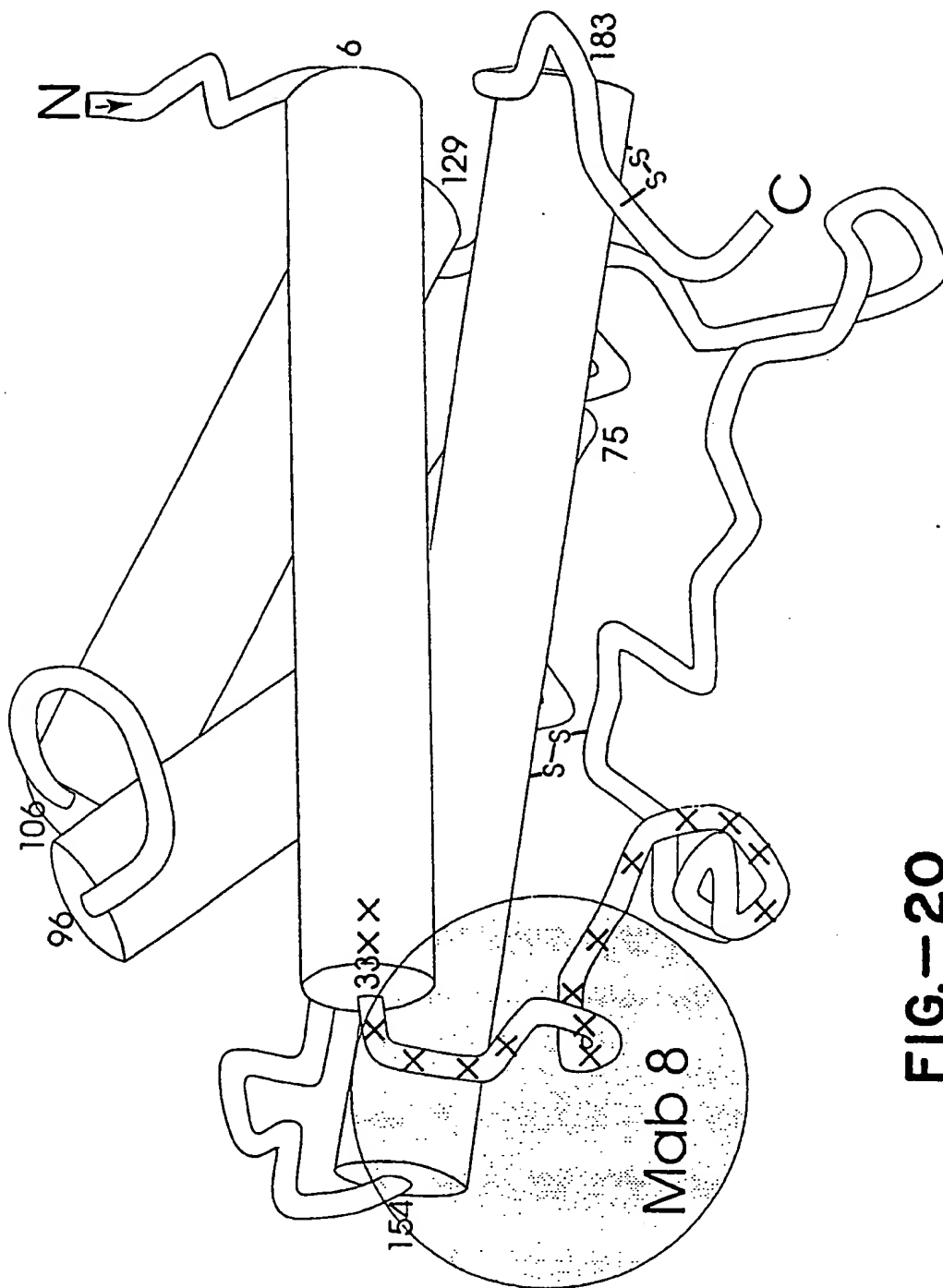
**FIG. 18**



**Fig. 19**

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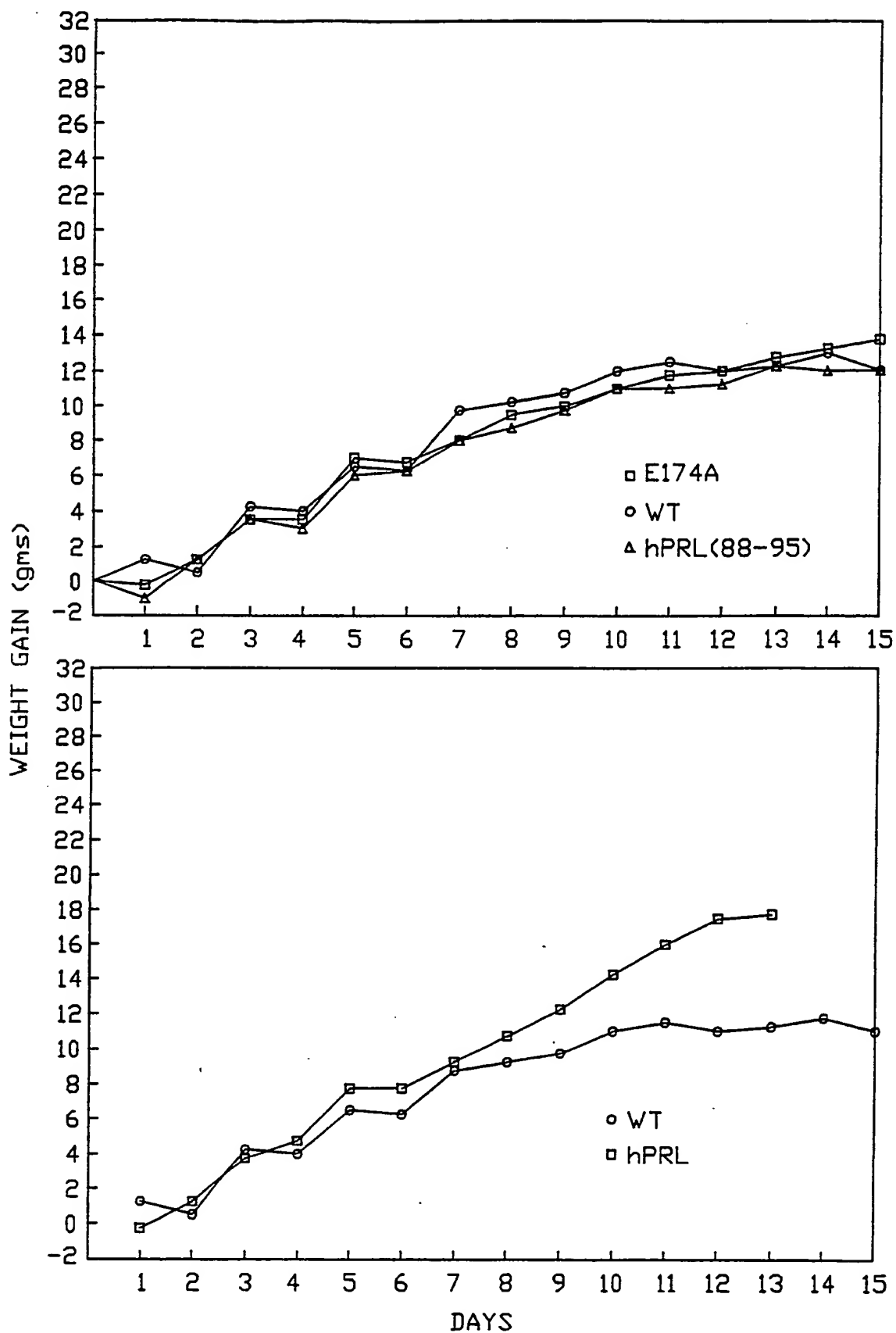
**FIG. -20**

X Deletion (32-46)

# SUBSTITUTE SHEET



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**FIG.-22**

SUBSTITUTE SHEET

## GH ANALOG BIO POTENCY IN RATS AFTER 8 DAYS OF TREATMENT

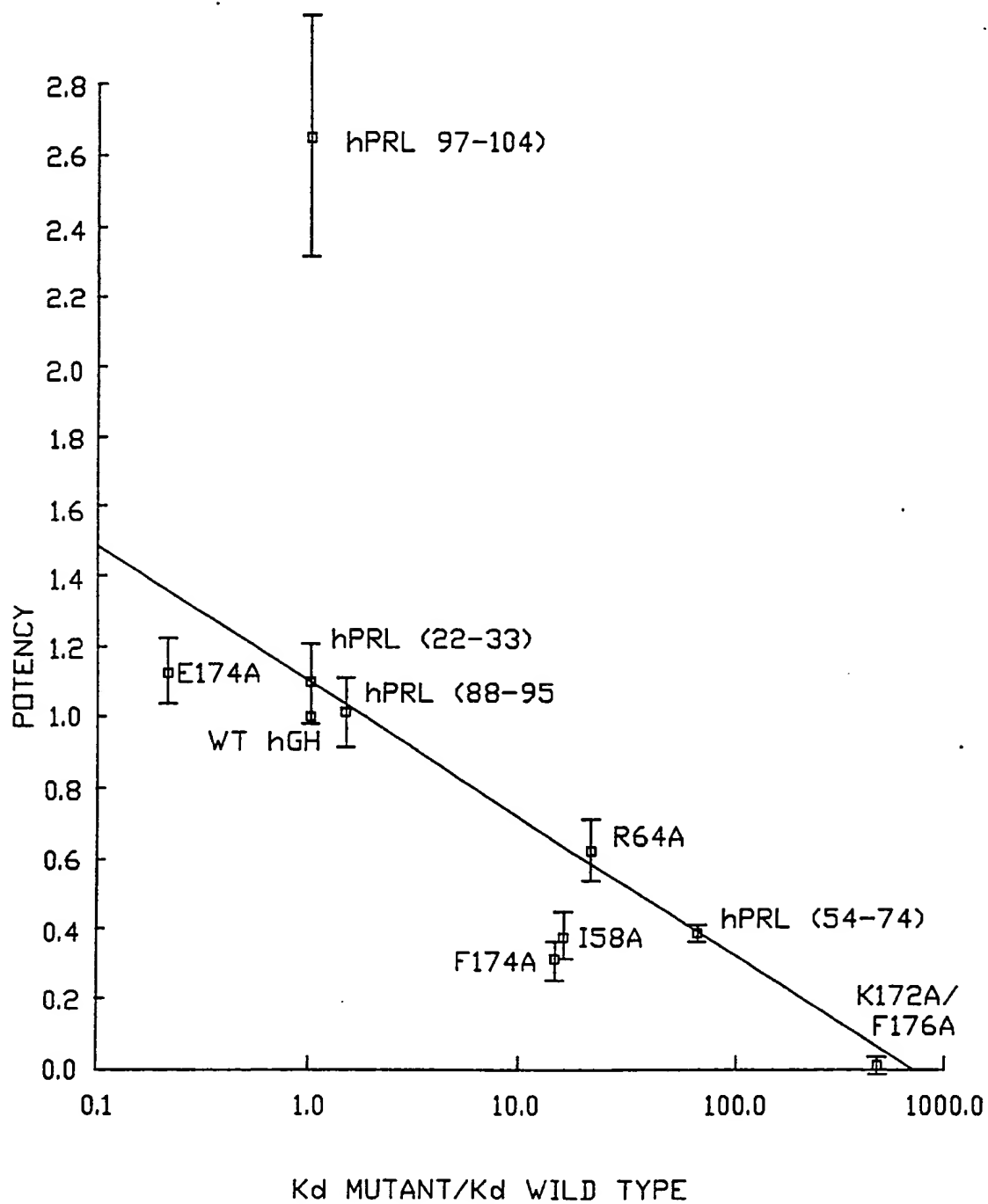


FIG.—23

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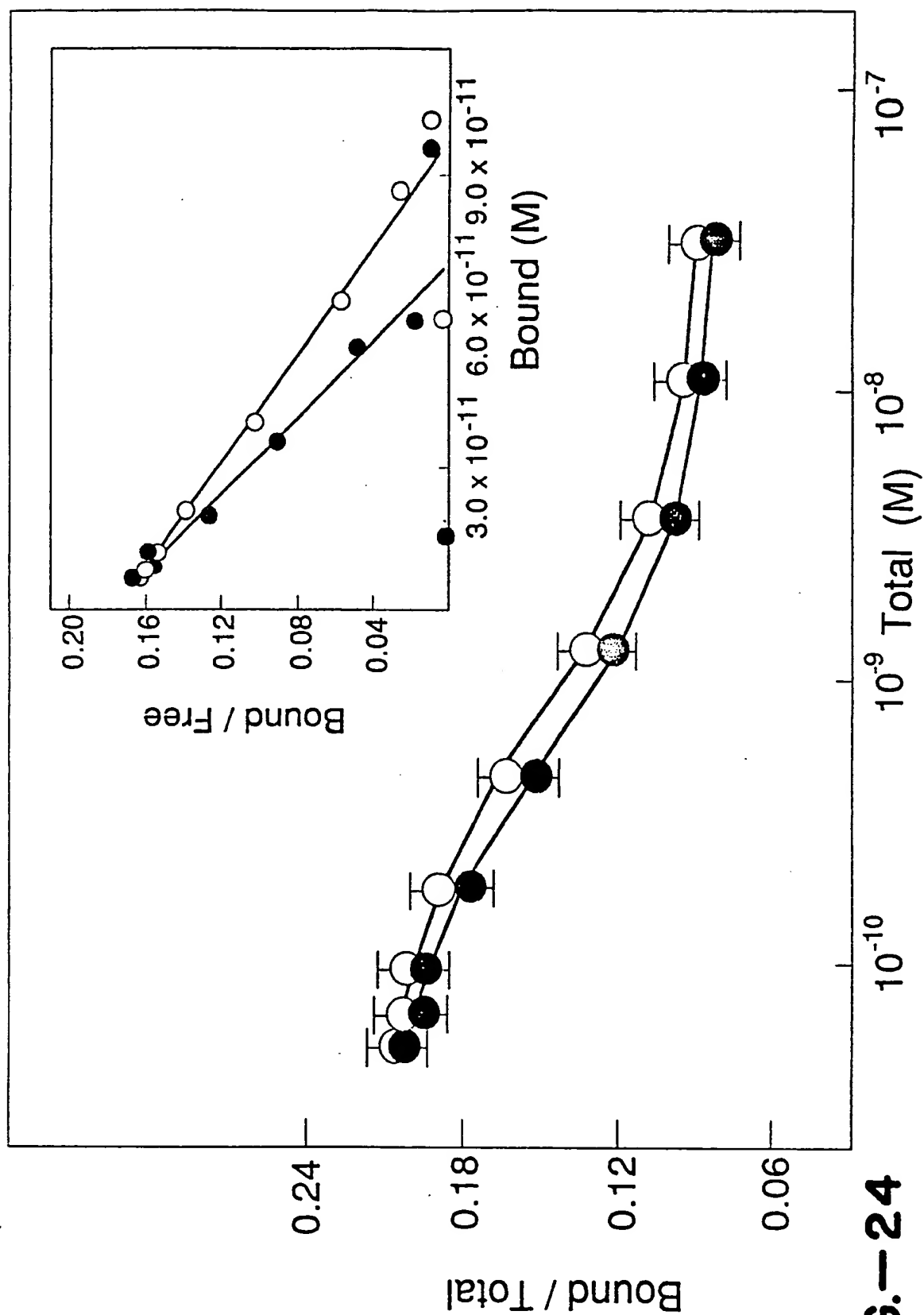


FIG.—24

SUBSTITUTE SHEET

## Binding Determinants for hGHR

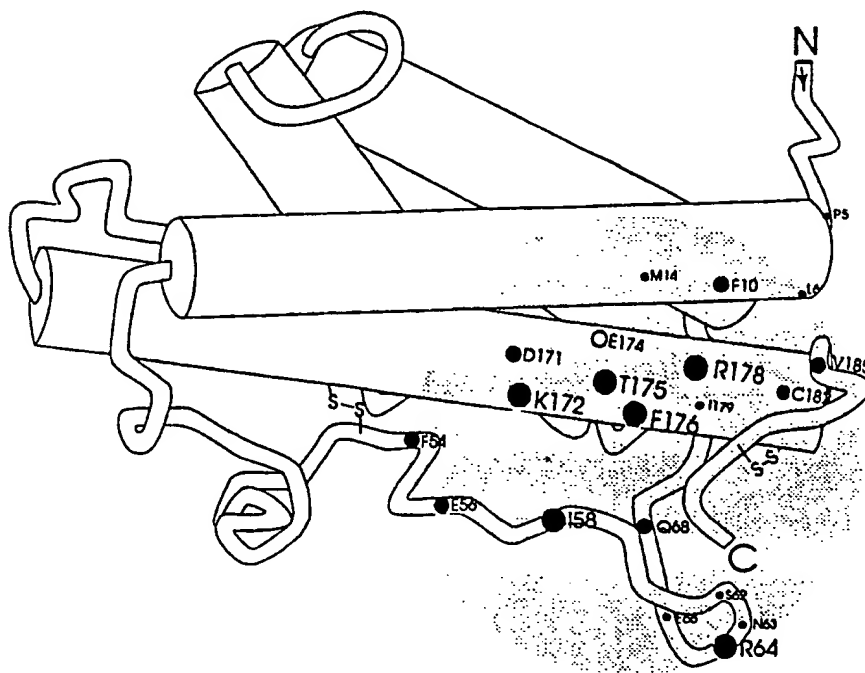


FIG.-25A

## Binding Determinants for hPRLr

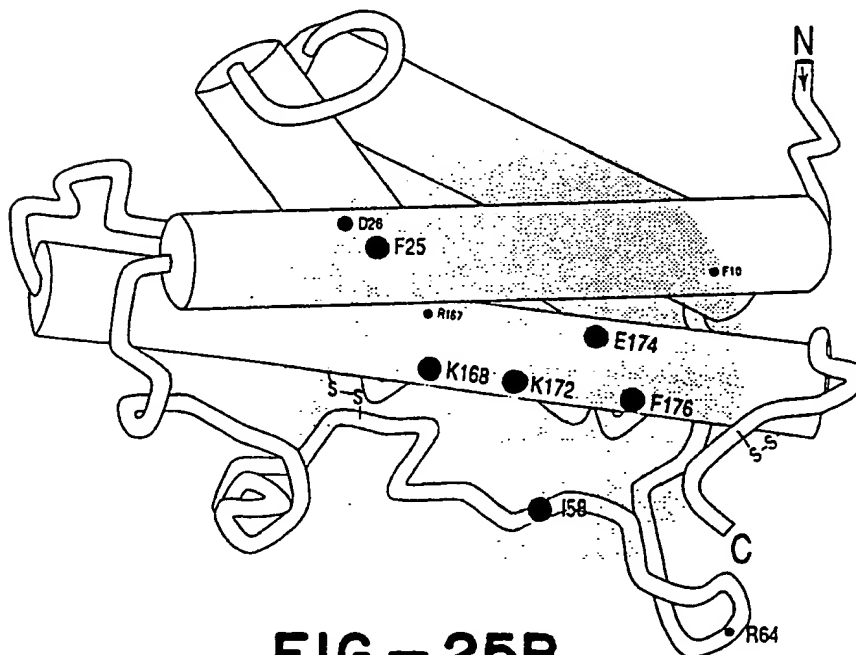


FIG.-25B

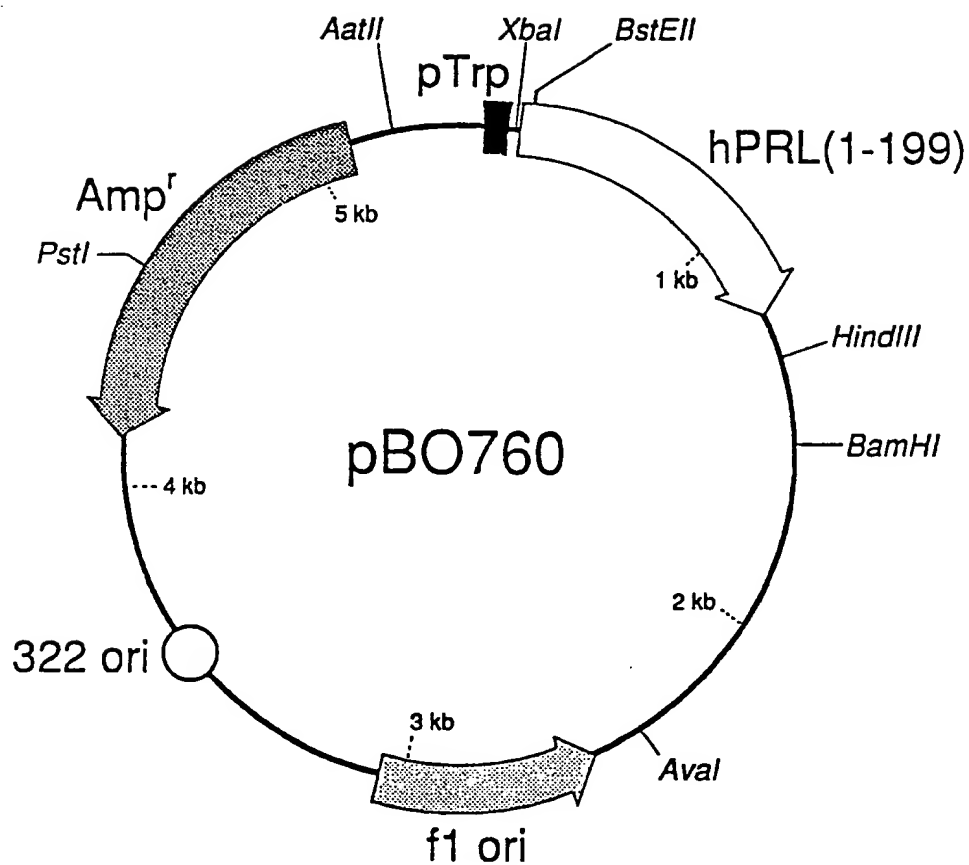


FIG.—26

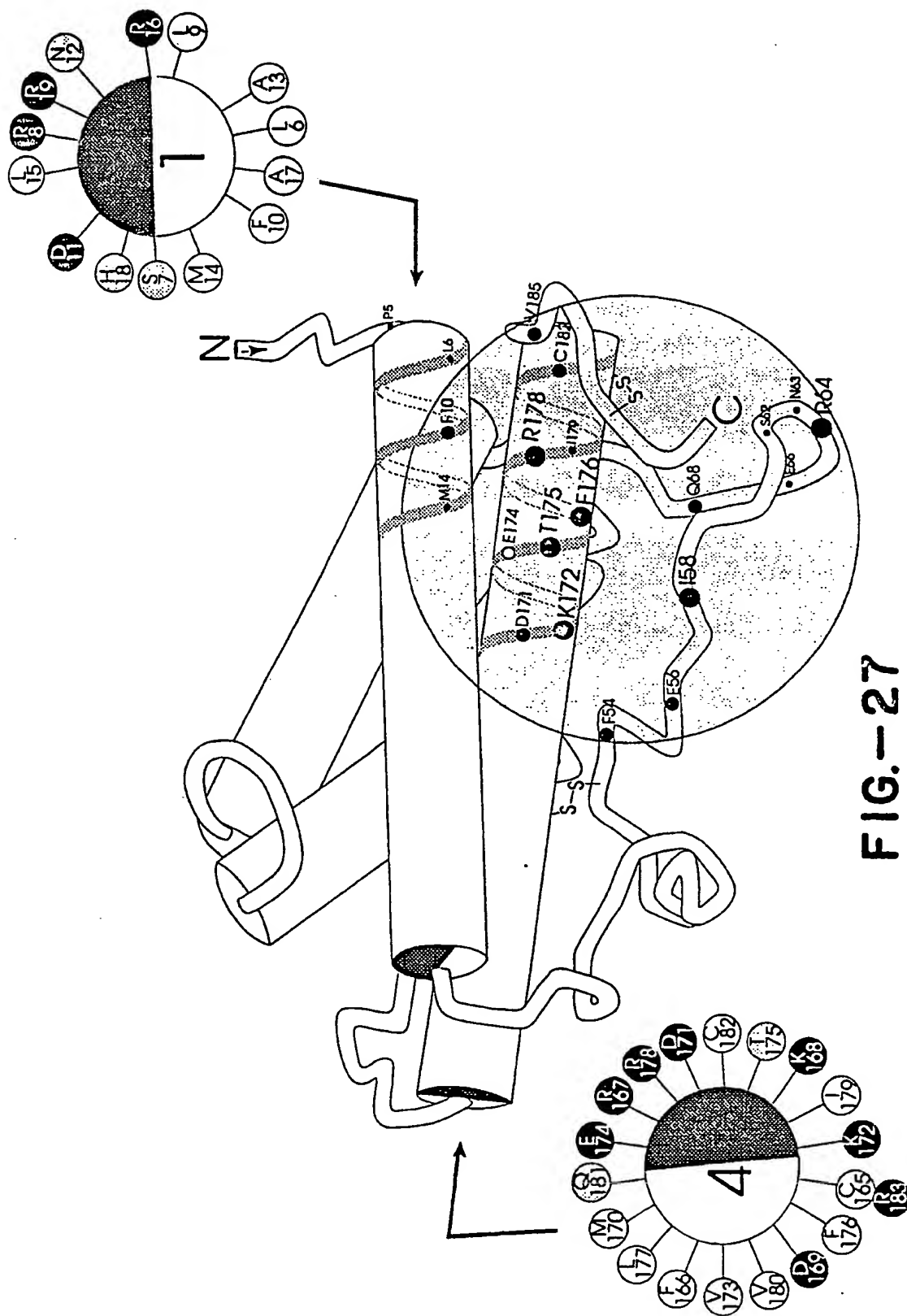
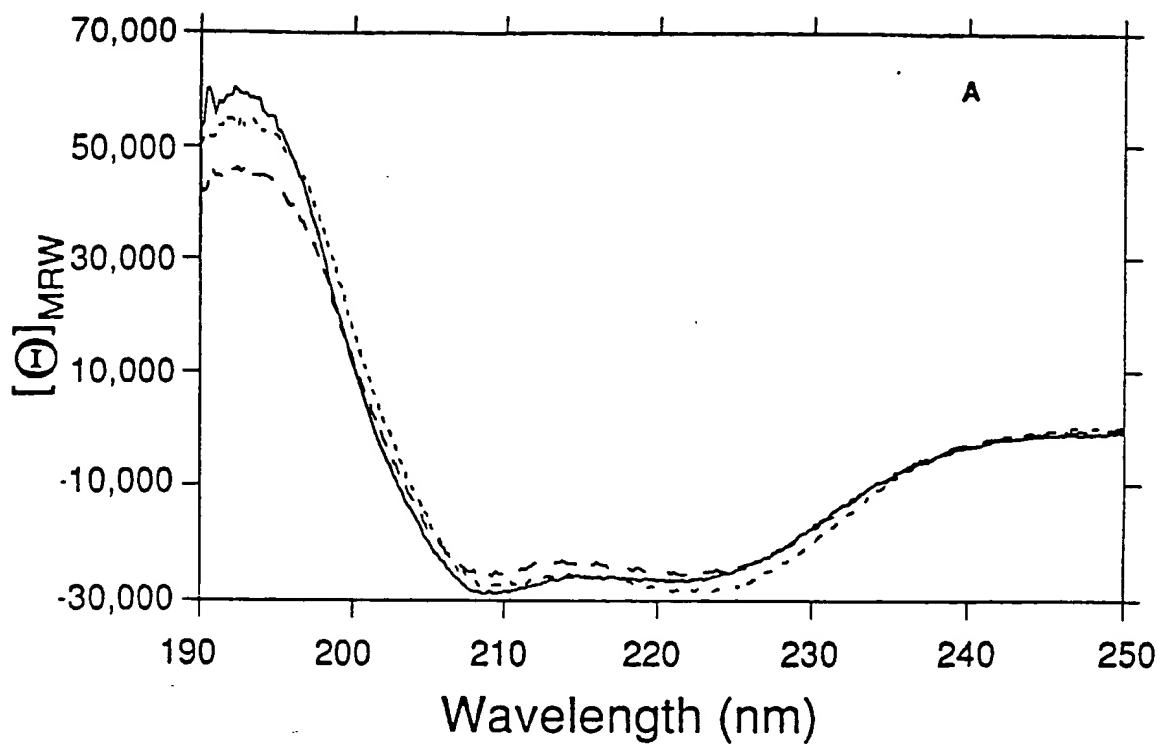
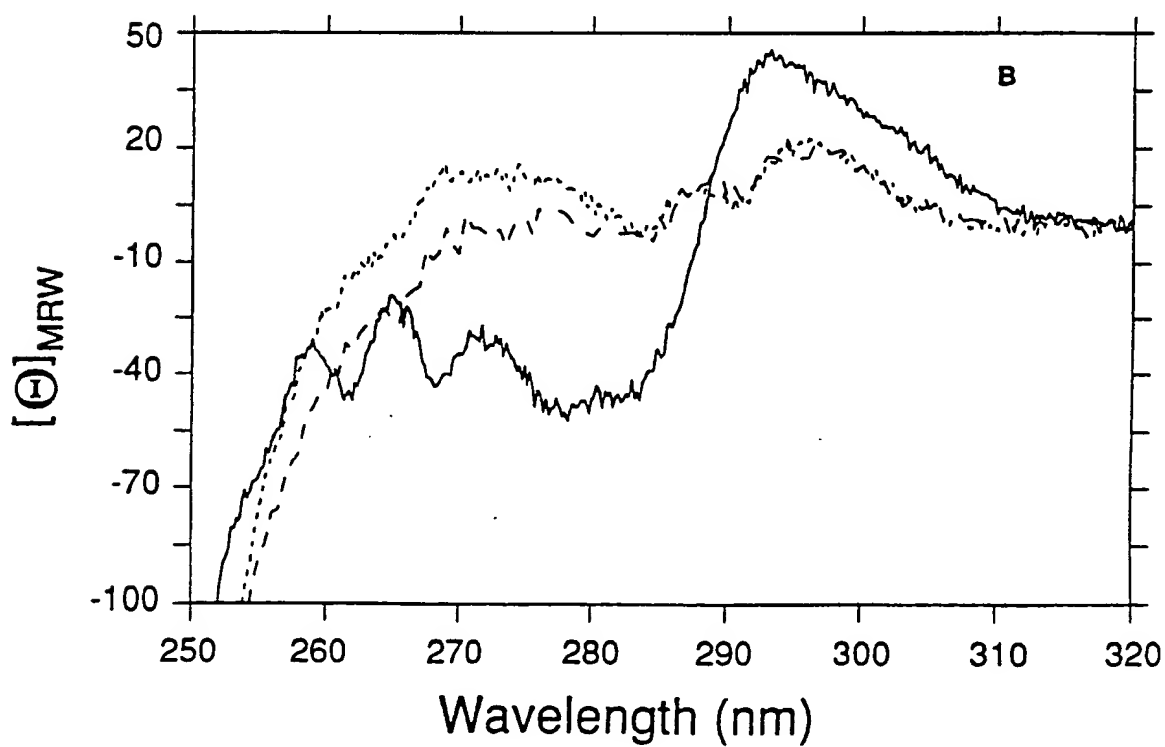


FIG.-27

**FIG.—28A****FIG.—28B**

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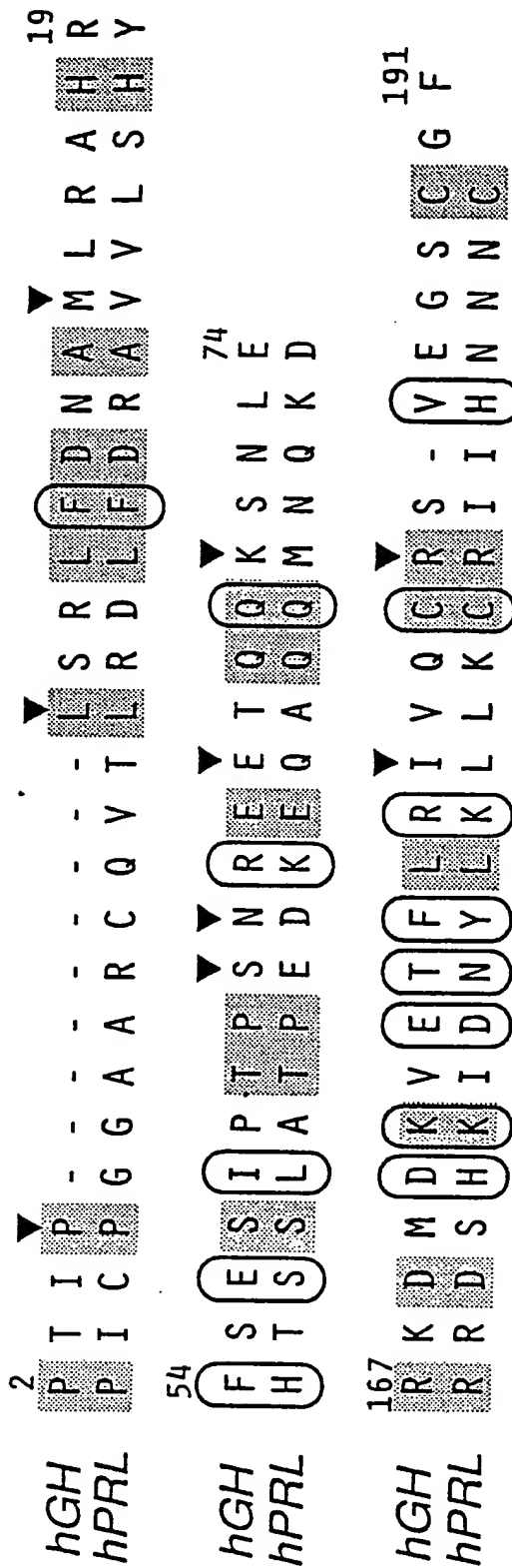


FIG.-29

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/04778

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC (5): G01N 33/53, 31/00, 33/543, 33/567, 33/566 U.S. Cl: 435/7; 436/501, 504, 518, 548		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
U.S.	435/7; 436/501, 504, 518, 548; 935/79, 81	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
CHEMICAL ABSTRACTS SERVICE ONLINE, BIOSIS PREVIEWS, AUTOMATED PATENT SYSTEM		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X <sub>5</sub> P	Science, Volume 244, issued 1989, CUNNINGHAM, B.C., ET AL, "High Resolution Epitope Mapping of hGH-Receptor Interactions by Alanine-Scanning Mutagenesis", 1081-1085.	1-13,16-31
X,P	Science, Volume 243, issued 1989, CUNNINGHAM, B.C., ET AL, "Receptor and Antibody Epitopes in Human Growth Hormone Identified by Homolog-Scanning Mutagenesis", 1330-1336.	1-13,16-31
A	Biochem. Biophys. Res. Commun., Volume 135, issued 1986, SOUROUTON, M.C., ET AL, "Localization of a Highly Immunogenic Region on the Acetylcholine Receptor Alpha-Subunit", 82-89.	1-13,16-31
<p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
02 February 1990	05 MAR 1990	
International Searching Authority	Signature of Authorized Officer	
ISA/US	KAREN I. KRUPEN	

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Endocrinol., Volume 121, issued 1987, WERTHER ET AL, "Localization and Characterization of Insulin Receptors in Rat Brain and Pituitary Gland Using In-Vitro Autoradiography and Computerized Densitometry, 1562-1570.	1-13,16-31
A	Endocrinology, Volume 107, issued 1980 MILLS, T.B. ET AL, "Fragments of human growth hormone produced by digestion with thrombin: chemistry and biological properties", 391-399 (See Abstract, 143544)	1-13,16-31
A	Chemical Abstracts, Volume 108, no. 11, issued 1988, (Columbus, Ohio, U.S.A) B. C. Cunningham, "Improvement in the alkaline stability of subtilisin using an efficient random mutagenesis and screening procedure", Abstract.	1-13,16-31



## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE<sup>1</sup>

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . . . . because they relate to subject matter<sup>12</sup> not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . . . because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out<sup>13</sup>, specifically:

3. ☐ Claim numbers . . . . . because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING<sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

(See Attachment).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1-13 and 16-31

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

ATTACHMENT TO PCT/ISA/210

Part IV. Before #1, Observations

- I. Claims 1-13 and 16-31 are drawn to a method for identifying unknown active domains in the amino acid sequence of polypeptides classified in class 436, subclass 501.
- II. Claims 14, 15 and 32-64 are drawn to a method of forming a growth hormone variant and the growth hormone variants produced classified in class 530, subclass 350.
- III. Claims 65-79 are drawn to human prolactin hormone variants classified in class 530, subclass 399.
- IV. Claims 80-83 are drawn to human placental lactogen variants classified in class 530, subclass 399.
- V. Claims 84-86 are drawn to DNA sequences and expression vectors and hosts classified in class 536, subclass 27.